Daily Notes

FRIDAY, 6/10/2016

or20MAGE: with Jaymin

I. Comp Cells

II. Transformation: Electroporate with Oligo

- Culture electrocompetent *E. coli* in liquid LB until ideal/midlog phase (Jaymin: OD 600 = 0.4 - 0.6 Moreno: OD 600 = 0.6 - 0.7)
  - For OD 600 = 1.0 ~ 1.0 x 10^9 cells
- carefully transfer cells to 1.7 ml eppendorf tubes -- add dH2O
- In cold room, centrifuge for 30 s x 10,000
- remove/"knock out" supernatant via flick method or pipette
- resuspend in 1 ml of dH2O
- centrifuge again for 30 s x 10,000
- resuspend in 1 ml of dH2O
- use dribble method to obtain approx. ? .1 mL (100)
- add 1? uL oligo
- pipette up and down 2x times then transfer ? xL//all of cells to center of electrocuvette between two electrodes
- electroporate at 18000* V
  - observe decay time, should be 5.0 - 5.2 seconds
- add ? xL of recovery fluid to electroporated cells in electrocuvette
- transfer cells to (25 mL) falcon tube with pipette
- allow 3 hrs recovery time to enable propogation of mutants (1st generation = 1/4 mutant, etc)

II. Rifampicin Assay

- Prepare rifampicin-50 or rif-100 with DMSO (concentration) , 500
  - can pipette with syringe luer-lock if want to use maintain sterility, but use of EtOH or DMSO as antibiotic solvent should preclude contamination
  - Carb-50, Kan-50, Spec-100, Rif-100 ** photo w specs
- 1000 concentration of Rif : 50 xL to 500 mL into LB liquid solution (autoclaved previously, agar added)
- mix throughly until all clumps are dissolved, pour plates

After 3 hours of recovery time (part I)

- pellet cells, remove excess H2O
- resuspend in 20 uL of water
- dispense into row A
- dispense 180 uL of buffer into well A
- pipette 180 uL of buffer into each well (rows B-G)
- transfer 20 uL from well A to well B, well B to C, etc. replacing tip ea time
- use glass beads to streak transformed cells at four serial dilutions (10^0, 10^-x, 10^-y 10^-6) on LB and rif+ (-50/-100) plates
  - 8 plates total for ea strain
- incubate at 36*C, observe for comparative growth on rif+ vs LB
  - growth on rif indicates MAGE successful in introducing point mutation to r3?? RNA polymerase gene, conferring spontaenous resistance
  - compare to negative control for background rate of spontanoues resistance

Eporation/Transformation Tricks: Colin's

wash/ resuspend 3x in water

milliQ (may not use for very fragile cells bc so pure absorbs CO2 from the air so is slightly acidic)

1st and 2nd suspension pipette up and down,

3rd time tap w finger bc cells are fragile

- eppendorf flicking/dumping technique does not work for smaller amounts of cells, safer to use pipettes

Jaymin will grow *R. tropici* CIAT 899 in Spec over the weekend to test resistance to spec (hopefully none observed so that we don't have to redesign/resequence the plasmid constructs w alt antibiotic

Isaacs Lab Ordering Google doc
There's an iGEM tab
https://docs.google.com/spreadsheets/d/1UxTuR2v-jTOvcPjDBdwm9PH3v8RXQggv6aSEVKDKcA0/edit#gid=14706218

MONDAY, 6/13/2016

Meeting w/ Farren
Slide one: I have been looking into the potential MAGE applications in *Synechococcus* sp. PCC7002 and one interesting aspect of it would be the production of UV-screening compounds.

Slide two: Our existing chemical sunscreens cause various health and environmental problems. Many cyanobacteria strands have the natural strength of producing a type of UV-screening compounds: Mycosporine-Like Amino Acid, i.e. MAAs. Many MAAs have simultaneous anti-oxidant properties in addition to their UV absorbing properties and thus may actually be more effective in preventing both UVA and UVB damage to human skins.

Slide three: The idea to use synthetic biology tools to optimize MAAs production has been explored by some iGEM teams in previous years. Baskus and Walsh (2010) identified a gene cluster in A. variabilis that produced shinorine. The U. Minnesota 2012 iGEM team successfully transformed the entire gene cluster but was able to observe any activities of the final enzyme of the pathway. The 2015 iGEM team of UCSF attempted a project where they identified 7 other homologous genes from known shinorine producing cyanobacteria and inserted plasmids with said genes into E. coli and applied guided evolution to maximize shinorine productions. They demonstrated that they produced UV absorbing compounds but were not able to characterize the production efficiency.

Slide four: Even though no current literature provides evidence that the strand PCC7002 itself produces shinorine, I think it is worthwhile to explore the possibility of PCC7002 serving as a model strand for shinorine production. First of all, MAA production involves rather complex biochemical pathway and to insert the entire pathway into E. coli is a very demanding project and the fact that PCC7002 is a cyanobacteria strand indicates that it is potentially more capable of MAA production than other microorganisms. At the same time, cyanobacterium are potentially more environmentally and economically beneficial because they are autotrophic and marine some marine stands (7002 included) do not even require freshwater for growth. Last but not least, with a doubling time of only 3.5h, 7002 is among the fastest-growing strains known. In a paper published in 2009, Singh and others performed genome mining in several cyanobacteria strands in the hope that they'd be able to identify MAA synthesizing pathways. The paper did not investigate 7002 as one of their strand but a similar process can be applied here. It is possible to perform gene mining in 7002 based on known MAA synthesis gene clusters or to insert such clusters from a closely related strand.

Slide five: Once we are able to either pinpoint the MAA synthesis pathway native to PCC 7002 or to insert gene clusters from related strands, MAGE would come into play and modify various enzymes in different steps of this pathway in the hope to optimize MAA production. One of the challenges facing the manipulation of 7002 is that there're no known entry vectors. There're 2 approaches to go around this: one would be either conjugative plasmid transfer from another strand; or employ 7002's natural competency in taking up linear DNAs. Either would incur and rather low transformation efficiency and would require a specific selection method because it takes a rather long period of time for 7002 to form colonies. One possible solution was described in a 2014 paper where it applies multiplex genome editing in cells that are known to be naturally competent, as a method to screen for cells that are ready to take up DNA. By cotransforming with an antibiotic resistance marker, one can effectively select for cells that have natural transformation turned "on." and then subject them to further selection with flow cytometer.

Email from Dr. Moreno
"I have to take my son tomorrow first thing in the morning to get some lab tests. I will in late in the morning. I will be happy to streak the PRO_CIT_TER constructs that were sequenced so you gave them on Tuesday."

>>pick-up strands on Tuesday

MAGE results from last Friday

after doing MAGE, you plated serial dilutions on plates with and without rifampicin to get colony counts (CFUs). The ratio of CFUs between the two plates gives you a conversion frequency.

Ryan's strain 1561 (EcNR2 enhanced Beta expression) was grown to OD 0.8, including a 90 minute heat induction at 42C.

Per reaction, 1mL of cells were washed 2x with cold sterile water and resuspended to a final volume of 50uL. 1uL of oligonucleotide (100uM stock concentration) was added and the cells were electroporated. After a 3 hour recovery, cells were serially diluted and 50uL were plated on LB and LB+100ug/mL rifampicin for CFU counts.

The oligonucleotide made a H526Y mutation in RNA polymerase

Table3

	A	B	C	D
1	Sample	CFUs on LB	CFUs on LB + Rif	Conversion Rate
2	Jinny	26x106	0.88x106	3.4%
3	Ying	22x106	2.10x106	9.5%
4	Zach	23x106	2.48x106	10.8%
5	No oligo control	168x106	33	1.9x10-7

Yale Box

Colin: Here's a link to the Yale box folder that Farren requested. This is Farren's favorite way of storing data online, so be sure to upload anything that he might want to look at to it.

https://yale.box.com/s/xr2c9yj6cbzyq1vqa1jkh13p71b4czny

Electrocomp cell prep protocol for rhizobia

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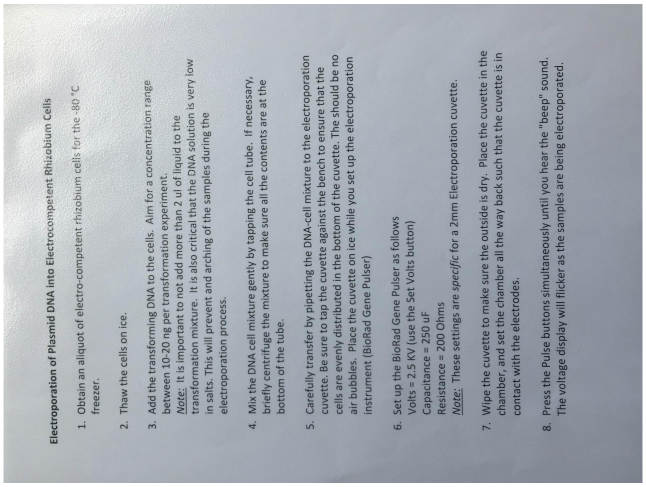

Electroporation of Plasmid DNA into Electrocompetent Rhizobium Cells

1. Obtain an aliquot of electro-competent rhizobium cells for the -80 °C freezer.
2. Thaw the cells on ice.
3. Add the transforming DNA to the cells. Aim for a concentration range between 10-20 ng per transformation experiment.
   Note: It is important to not add more than 2 ul of liquid to the transformation mixture. It is also critical that the DNA solution is very low in salts. This will prevent and arching of the samples during the electroporation process.
4. Mix the DNA cell mixture gently by tapping the cell tube. If necessary, briefly centrifuge the mixture to make sure all the contents are at the bottom of the tube.
5. Carefully transfer by pipetting the DNA-cell mixture to the electroporation cuvette. Be sure to tap the cuvette against the bench to ensure that the cells are evenly distributed in the bottom of the cuvette. The should be no air bubbles. Place the cuvette on ice while you set up the electroporation instrument (BioRad Gene Pulser)
6. Set up the BioRad Gene Pulser as follows
   Volts = 2.5 KV (use the Set Volts button)
   Capacitance = 250 uF
   Resistance = 200 Ohms
   Note: These settings are *specific* for a 2mm Electroporation cuvette.
7. Wipe the cuvette to make sure the outside is dry. Place the cuvette in the chamber, and set the chamber all the way back such that the cuvette is in contact with the electrodes.
8. Press the Pulse buttons simultaneously until you hear the "beep" sound. The voltage display will flicker as the samples are being electroporated.

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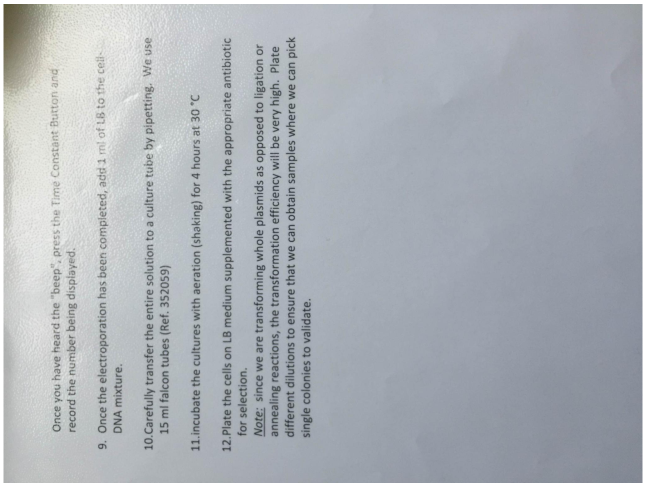

Once you have heard the "beep", press the Time Constant Button and record the number being displayed.

9. Once the electroporation has been completed, add 1 ml of LB to the cell-DNA mixture.

10.Carefully transfer the entire solution to a culture tube by pipetting. We use 15 ml falcon tubes (Ref. 352059)

11.Incubate the cultures with aeration (shaking) for 4 hours at 30 °C

12.Plate the cells on LB medium supplemented with the appropriate antibiotic for selection.
Note: since we are transforming whole plasmids as opposed to ligation or annealing reactions, the transformation efficiency will be very high. Plate different dilutions to ensure that we can obtain samples where we can pick single colonies to validate.

Primers for sequencing citrine

**Gloria put them in -80. Should be kept at -20.

- Find out where it is now and label [6/14/15 LYW]

Table4

	A	B	C	D	E	F
1	No.	ID	Name	Sequence	Scale	Purification
2	1	priG351	Ara-F	cactcagggagagatctgatgaa	25nm	STD
3	2	priG352	Mel-F	agctcttcaccgacattctttc	25nm	STD
4	3	priG353	Rha-F	gcgcatgcacgagaaag	25nm	STD
5	4	priG354	Tau-F	acataaccaaagagggtaacgac	25nm	STD
6	5	priG356	Citrine-R	cgactgagcctttcgttttatttg	25nm	STD
7	6	priG357	Up-citrine-F	gcatacgctacttgcattacag	25nm	STD
8	7	priG358	Up-citrine-R	tcgcccttgctcaccat	25nm	STD
9	8	priG359	Down-citrine-F	caaggaggacggcaacatc	25nm	STD
10	9	priG360	Down-citrine-R	acgacaatctgatccaagctc	25nm	STD
11	10	priG361	Astr-F	gctagctcagtcctaggtaca	25nm	STD
12	11	priG362	Amed-F	gctagctcagtcctaggtatagt	25nm	STD
13	12	priG363	Awek-F	gctagctcagtcctaggtacaat	25nm	STD

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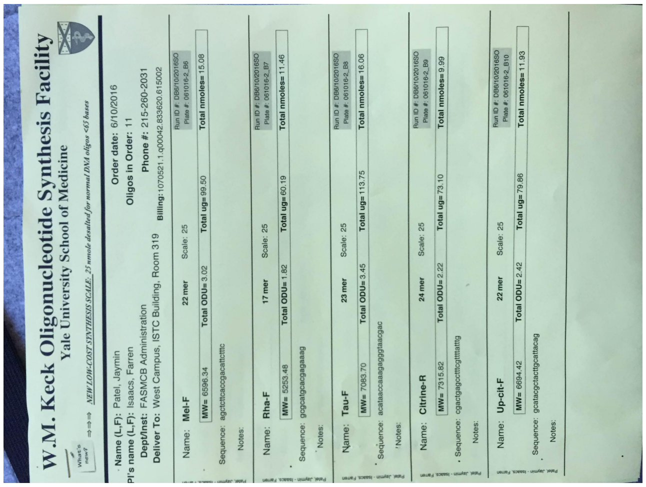

W.M. Keck Oligonucleotide Synthesis Facility
Yale University School of Medicine

What's new? ⇒⇒⇒ NEW LOW-COST SYNTHESIS SCALE: 25 nmole desalted for normal DNA oligos <65 bases

Name (L,F): Patel, Jaymin
PI's name (L,F): Isaacs, Farren
Dept/Inst: FASMCB Administration
Deliver To: West Campus, ISTC Building, Room 319
Order date: 6/10/2016
Oligos in Order: 11
Phone #: 215-260-2031
Billing: 1070521.1.q00042.833620.615002

Name: Mel-F 22 mer Scale: 25 Run ID #: DB6/10/2016SO Plate #: 061016-2_B6
MW= 6596.34 Total ODU= 3.02 Total ug= 99.50 Total nmoles= 15.08
Sequence: agctcttcaccgacattctttc
Notes:

Name: Rha-F 17 mer Scale: 25 Run ID #: DB6/10/2016SO Plate #: 061016-2_B7
MW= 5253.48 Total ODU= 1.82 Total ug= 60.19 Total nmoles= 11.46
Sequence: gcgcatgcacgagaaag
Notes:

Name: Tau-F 23 mer Scale: 25 Run ID #: DB6/10/2016SO Plate #: 061016-2_B8
MW= 7083.70 Total ODU= 3.45 Total ug= 113.75 Total nmoles= 16.06
Sequence: acataaccaaagaggtaacgac
Notes:

Name: Citrine-R 24 mer Scale: 25 Run ID #: DB6/10/2016SO Plate #: 061016-2_B9
MW= 7315.82 Total ODU= 2.22 Total ug= 73.10 Total nmoles= 9.99
Sequence: cgactgagcctttcgttttatttg
Notes:

Name: Up-cit-F 22 mer Scale: 25 Run ID #: DB6/10/2016SO Plate #: 061016-2_B10
MW= 6694.42 Total ODU= 2.42 Total ug= 79.86 Total nmoles= 11.93
Sequence: gcatacgctacttgcattacag
Notes:

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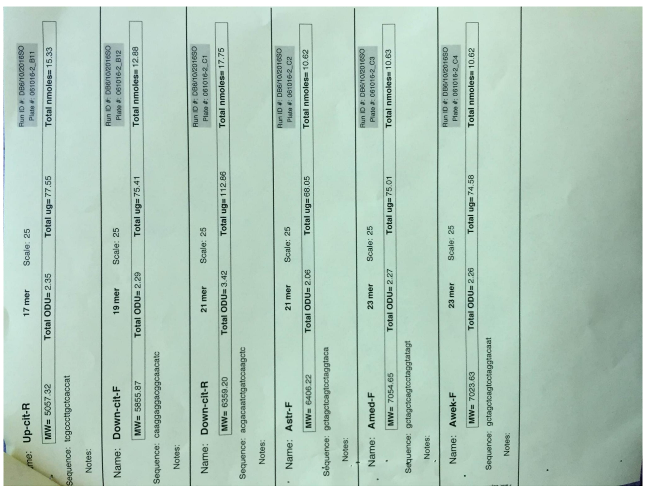

Name: Up-cit-R 17 mer Scale: 25 Run ID #: DB6/10/2016SO Plate #: 061016-2_B11
MW= 5057.32 Total ODU= 2.35 Total ug= 77.55 Total nmoles= 15.33
Sequence: tcgcccttgctcaccat
Notes:

Name: Down-cit-F 19 mer Scale: 25 Run ID #: DB6/10/2016SO Plate #: 061016-2_B12
MW= 5855.87 Total ODU= 2.29 Total ug= 75.41 Total nmoles= 12.88
Sequence: caaggaggacggcaacatc
Notes:

Name: Down-cit-R 21 mer Scale: 25 Run ID #: DB6/10/2016SO Plate #: 061016-2_C1
MW= 6359.20 Total ODU= 3.42 Total ug= 112.86 Total nmoles= 17.75
Sequence: acgacaatctgatccaagctc
Notes:

Name: Astr-F 21 mer Scale: 25 Run ID #: DB6/10/2016SO Plate #: 061016-2_C2
MW= 6406.22 Total ODU= 2.06 Total ug= 68.05 Total nmoles= 10.62
Sequence: gctagctcagtcctaggtaca
Notes:

Name: Amed-F 23 mer Scale: 25 Run ID #: DB6/10/2016SO Plate #: 061016-2_C3
MW= 7054.65 Total ODU= 2.27 Total ug= 75.01 Total nmoles= 10.63
Sequence: gctagctcagtcctaggtatagt
Notes:

Name: Awek-F 23 mer Scale: 25 Run ID #: DB6/10/2016SO Plate #: 061016-2_C4
MW= 7023.63 Total ODU= 2.26 Total ug= 74.58 Total nmoles= 10.62
Sequence: gctagctcagtcctaggtacaat
Notes:

TUESDAY, 6/14/2016

Tasks from Jaymin's email

1. Someone needs to check in on the portmage transformations.
2. We need to begin transforming the citrine constructs into rhizo. This may involve making comp cells out of meliloti.
3. The citrine constructs in e coli need to be brought to west campus for testing
4. We will be testing portmage in e coli tomorrow
    Someone needs to transport the strain to west campus for this
    Someone needs to make some rifampicin and lb plates
5. Of the messed up recombinase constructs, someone needs to pick additional colonies for sequencing
6. If dr. Moreno is going to begin starting plants for nodulation assays, someone should be there to shadow and take detailed notes on the process.

Brought some tubes and plates from OML to WC

(tubes [LYW 6/14/16] stored at -20 C, plates [LYW 6/14/16] stored at 4 C)

1. single colonies of entry vectors (Promoter_SacB_Terminator)
2. Plasmid DNA of entry vectors (Promoter_SacB_Terminator)
3. Plasmid DNA of citrine cinstructs (Promoter_Citrine_Terminator)
4. Plasmid DNA of pORTMAGE (two different colonies made by Gloria and Mindy)

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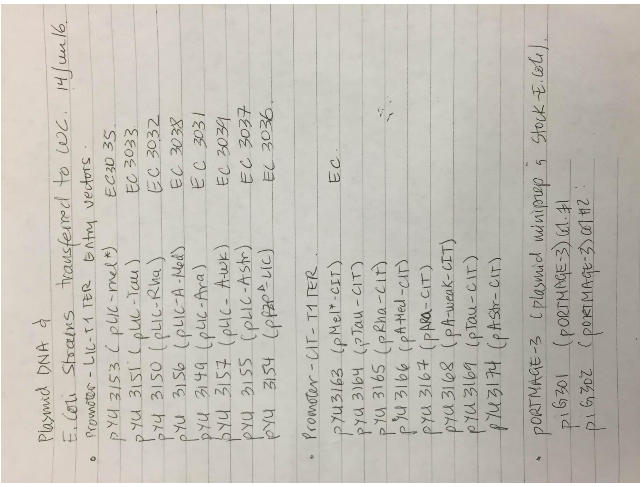

Plasmid DNA &
E. Coli Strains transferred to LOC. 14/Jun/16.
• Promoter-LIC-T1 TER Entry vectors.
pYU 3153 (pLIC-mel*) EC3035
pYU 3151 (pLIC-Tau) EC 3033
pYU 3150 (pLIC-Rha) EC 3032
pYU 3156 (pLIC-A-Mel) EC 3038
pYU 3149 (pLIC-Ara) EC 3031
pYU 3157 (pLIC-Awk) EC 3039
pYU 3155 (pLIC-Astr) EC 3037
pYU 3154 ([illegible] LIC) EC 3036.
• Promoter-CIT-T1 TER. EC.
pYU3163 (pMel*-CIT)
pYU 3164 (pTau-CIT)
pYU 3165 (pRha-CIT)
pYU 3166 (pA-Mel-CIT)
pYU 3167 (pARA-CIT)
pYU 3168 (pA-weak-CIT)
pYU 3169 (pTau-CIT)
pYU3174 (pAStr-CIT)
• pORTMAGE-3 (plasmid miniprep & stock E. coli).
pi6301 (pORTMAGE-3) col.#1
pi6302 (pORTMAGE-3) col#2.

Ordered Primers for sequencing pPRTMAGE [ordered by Colin]
file name: 6/14/16- Primer Order for pORTMAGE sequencing
* Dr. Moreno wants the primers in OML by tomorrow (Wed, June 15th)

Table1

	A	B	C	D	E	F	G	H
1	1	priG364	Gam-F	ATGGATATTAATACTGAAACTGAGATCAAG	25nm	STD	GJW	For verification of pORTMAGE
2	2	priG365	Gam-R	TTATACCTCTGAATCAATATCAACCTGG	25nm	STD	GJW	For verification of pORTMAGE

Sequencing reactions
14 uL of DNA w/ concentration of 40 ng/uL + 1 uL of Primer w/ concentration of 5-15 ng/uL (original concentration: 20 ng/uL)
filled up the volume w/ MilliQ water
number PCR tubes*8 from 1 to 14 and send in the tubes by 4pm

Table2

	A	B	C	D	E	F	G	H	I
1	PCR Tube No.	Plasmid Name	Plasmid ID	DNA Concentration ng/uL	Primer Name	Primer ID	Primer Concentration uM	DNA Volume added uL	Primer Volume added uL
2	1	pMel-CIT	pYU 3163	273.5	Mel-F	priG352	5	2.05	1
3	2	pMel-CIT	pYU 3163	273.5	Citrine-R	priG356	5	2.05	1
4	3	pRha-CIT	pYU3165	286.5	Rha-F	priG353	5	1.95	1
5	4	pRha-CIT	pYU3165	286.5	Citrine-R	priG356	5	1.95	1
6	5	pAra-CIT	pYU3167	336.5	Ara-F	priG351	5	1.66	1
7	6	pAra-CIT	pYU3167	336.5	Citrine-R	priG356	5	1.66	1
8	7	pTau-CIT	pYU3169	281	Tau-F	priG354	5	1.99	1
9	8	pTau-CIT	pYU3169	281	Citrine-R	priG356	5	1.99	1
10	9	pA-Med-CIT	pYU3166	257	Amed-F	priG362	5	2.18	1
11	10	pA-Med-CIT	pYU3166	257	Citrine-R	priG356	5	2.18	1
12	11	pA-Weak-CIT	pYU3168	134	Awek-F	priG363	5	4.18	1
13	12	pA-Weak-CIT	pYU3168	134	Citrine-R	priG356	5	4.18	1
14	13	pA-Str-CIT	pYU3174	251	Astr-F	priG361	5	2.23	1
15	14	pA-Str-CIT	pYU3174	251	Citrine-R	priG356	5	2.23	1

**Primer Ara-F was missing. There might be something wrong / the order. Emailed Jaymin about this.

Putting Primers in Tris Buffers
100x Tris diluted with MQ Water. Run the diluted buffer through antibacterial syringe. Primers ordered from Keck come in as 25nm(nanomole) per tube. Add one mL of 1x Tris and then dilute the tube contents to 1/5.

Making Glycerol Stocks for the entry vectors (Promoter_SacB_Terminator)
- Streak single colonies
- Pick a colony added to 3mL LB + 3uL of 100 mg/mL spec
- grow until turbid

LB Plates & Media
Zach made two sleeves of LB plates w/ spec, two sleeves w/ rip. Left on the bench.
- Need to be marked and put into 4 C.

One LB bottle was obtained from Jaymin and dispensed into three different small bottles and reautoclaved.
- Need to be taken out and marked.

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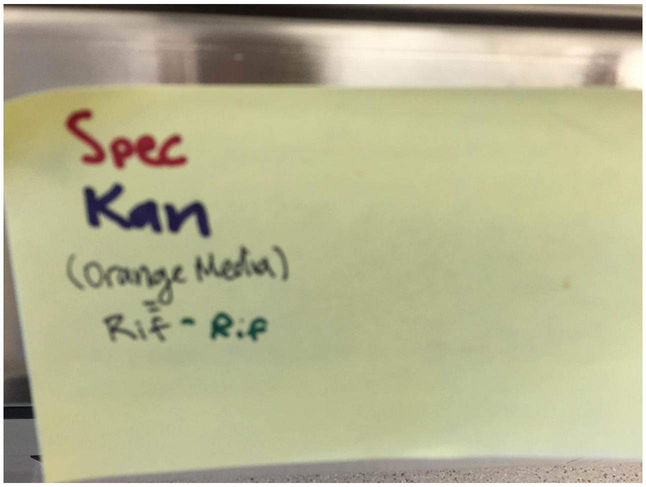

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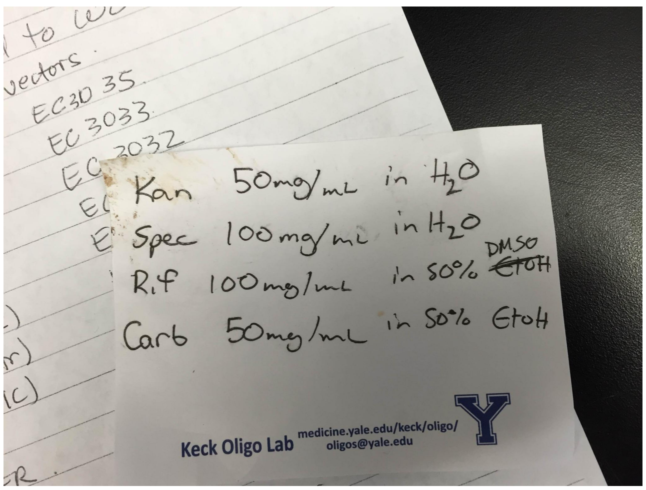


G-Blocks

Jaymin: errors were likely introduced during the cloning/sequencing rxn rather than in the actual IDT gBLOCK

IDT is very accurate

introducing into a topo vector and adding cloning sites is unnecessarily complicated and roundabout

just put directly into the vector

- Ask Dr. Moreno about obtaining the library.
- or, ask Dr. Moreno about designing the primers for point mutagenisis

  Site directed mutagenesis: use a mutagenic primer (forward and reverse) to clone the vector (but the vectors are long? eh)

use **Dpn1 to selectively degrade template plasmid/DNA; make a small 300 bp overlap for assay of correct replacement of the base

Suicide Vector for knocking out MutS in Rhizo

- read the paper before Friday (http://www.sciencedirect.com/science/article/pii/0378111993906116 (http://www.sciencedirect.com/science/article/pii/0378111993906116))
- Email Natalie back!!!

Jaymin's idea on testing portmage

Wild-type e.coli + oligo
E. coli w/ MAGE + oligo
E. coli w/ portmage + oligo
Wild-type rhizo + oligo
Rhizo w/ pprtmage + oligo ???

96 well for fluorescence assay
一个promoter一行，最后一行放negative control
每一行serial dilution

WEDNESDAY, 6/15/2016

Pick op colonies from Dr. Moreno

- Transformed e. coli w/ portmage [LYW 6/15/16 @ 4C]
- Glycerol stocks of citrine constructs [LYW 6/15/16 @ -80C]

** vortex glycerol stocks before freezing: to resuspend

Missing primer
The missing primer was received on Tuesday.

- Add Tris buffer and add the primer to sequencing reaction.
- Send off the sequencing reaction.

Taurine order

- emailed Colin about the delayed order. Waiting for response.
    - Colin says ask Amy about it
        - Amy will place the order this afternoon (Wed 6/15/16)
- find out about other small molecules

G-Blocks

- [ ] Gloria will ask Dr. M if we could get the tubes containing GBlocks

Prep stock solution for primers
The sheet that cme w/ the primers have moles marked under each entry
stock solution: 100 micro molar; working solution: 10 micro molar

Genewiz Sequencing Guidelines

Screen Shot 2016-06-15 at 4.01.56 PM.png

DNA Type	DNA Length (include vector)	Template Concentration in 10 µl	Template Total Mass	Your Primer Total Picomoles	Premixed Volume* (Template + Your Primer)
Plasmids	<6 kb	~50 ng / µl	~500 ng	25 pmol	15 µl
	6 - 10 kb	~80 ng / µl	~800 ng		
	> 10 kb	~100 ng / µl	~1000 ng		
Purified PCR Products	<500 bp	~1 ng / µl	~10 ng	25 pmol	15 µl
	500 - 1000 bp	~2 ng / µl	~20 ng		
	1000 - 2000 bp	~4 ng / µl	~40 ng		
	2000 - 4000 bp	~6 ng / µl	~60 ng		
	> 4000 bp	Treat as plasmid	Treat as plasmid		

Remade Sequencing Reactions

Table5

	A	B	C	D	E	F	G	H	I	J	
1	PCR Tube No.	Plasmid Name	Plasmid ID	DNA Concentration ng/uL	Primer Name	Primer ID	Primer Concentration	DNA Volume added uL	Primer Volume added uL	dH2O volume added uL	To Vc uL
2	1	pMel-CIT	pYU 3163	273.5	Mel-F	priG352		2.05	2.5	10.45	
3	2	pRha-CIT	pYU3165	286.5	Rha-F	priG353		1.95	2.5	10.55	
4	3	pMel-CIT	pYU 3163	273.5	Citrine-R	priG356		2.05	2.5	10.45	
5	4	pRha-CIT	pYU3165	286.5	Citrine-R	priG356		1.95	2.5	10.55	
6	5	pAra-CIT	pYU3167	336.5	Ara-F	priG351		1.66	2.5	10.84	
7	6	pAra-CIT	pYU3167	336.5	Citrine-R	priG356		1.66	2.5	10.84	
8	7	pTau-CIT	pYU3169	281	Tau-F	priG354		1.99	2.5	10.51	
9	8	pTau-CIT	pYU3169	281	Citrine-R	priG356		1.99	2.5	10.51	
10	9	pA-Med-CIT	pYU3166	257	Amed-F	priG362		2.18	2.5	10.32	
11	10	pA-Med-CIT	pYU3166	257	Citrine-R	priG356		2.18	2.5	10.32	
12	11	pA-Weak-CIT	pYU3168	134	Awek-F	priG363		4.18	2.5	8.32	
13	12	pA-Weak-CIT	pYU3168	134	Citrine-R	priG356		4.18	2.5	8.32	
14	13	pA-Str-CIT	pYU3174	251	Astr-F	priG361		2.23	2.5	10.27	
15	14	pA-Str-CIT	pYU3174	251	Citrine-R	priG356		2.23	2.5	10.27	

***Notice No. 2 and No. 3 were switched from the original form.

- [ ] Jaymin sent them off for sequencing.

MAGE in E.coli & Rhizo w/ Jaymin

1. Inude the strand for 1.5h in 42C water bath, take 1mL in Eppendrf tube, take to the cold room (4 C)
   **aliquot* (A representative sample of a fixed proportion. Thus 50l from a 25ml solution represents 1/500 of the total),
2. spin down (speed: 10000; time: 40s) and do 2 washes (add 1mL water, dump out the supernatant for the first wash and dribble out the supernatant for the last to leave ~50uL water in the tube) pipet up and down to resuspend the cells in ~50uL water
   *CIAT is more fragile than e. coli: be careful pipetting up and down; no dumpdown after the 2 washes
   *** [Dr. Moreno diagrees] Save comp cell in 10% glycerol, but for now water is good
3. add 1 uL of 100 microM oligo/pORTMAGE to each Eppendrf tube;
   a. Oligo names: rpoB- e. coli; CIAT: CIAT
4. Electroporation:
   a. Setting: e.coli: 1800, 25, 200, 1; rhizo: 2500, 250*, 200, 2.
      * we couldn't get as high as 250 so we did 50 but the cells were electrocuted [sharp beep from the machine]
   b. Cuvette: for e. coli: 1uL gap; for rhizo: 2 uL gap
   c. Time constant: >= 5; otherwise too much salt, the cells might be shocked
5. Cell Recovery

a. Incubate e. coli in 34 C shaking for 3 hours (double the normal recobery time, bc the lagging strand mechanism of MAGE)
b. Incubate rhizo in 28 C for overnight

**Jaymin had 10 times as much cells for rhizo-pORTMAGE transformation as it supposed be to (bc pORTMAGE transformation rate is pretty low) so we probably should have done more than 2 washes to get rid of all the cells

Glycerol Stock

Made 100 mL 80% glycerol [LYW 6/15/16] and 100 mL 10% glycerol [LYW 6/15/16] for future use.

- [ ] Wait for Jaymin to see how to keep sterile

Suicide Vector for knocking out MutS in Rhizo

- read the paper before Friday (http://www.sciencedirect.com/science/article/pii/0378111993906116 (http://www.sciencedirect.com/science/article/pii/0378111993906116))
- Email Natalie back!!!

Jaymin's Rhizo Antibiotic Resistance Data

"I have the data on antibiotic resistance in CIAT and Meliloti. In the table below, I've listed the Mean Inhibitory Concentrations (MIC) in ug/mL of each antibiotic (this refers to the concentration of antibiotic that inhibits bacterial growth - so the lower the number, the more sensitive the strain is to the antibiotic)."
"Ultimately, in Meliloti, you cannot use Streptomycin, and need to use a higher concentration of Kanamycin (using neomycin instead might improve results). In CIAT, you cannot use spectinomycin, streptomycin, or zeocin."

Table6

	A	B	C	D	E
1	Antibiotic	MIC Meliloti	Recommended Concentration Meliloti	MIC CIAT	Recommended Concentration CIAT
2	Carbenicillin	<2.5	50	10	50
3	Kanamycin	50	100	<2.5	35
4	Spectinomycin	25	100	>100	Do Not Use
5	Streptomycin	>100	Do Not Use	>100	Do Not Use
6	Tetracycline	<2.5	7.5	<2.5	7.5
7	Rifampicin	<2.5	100	<2.5	100
8	Gentamycin	10	50	<2.5	10
9	Zeocin	<2.5	7.5	>100	Do Not Use
10	Hygromycin	5	25	<2.5	25
11	Erythromycin	<2.5	25	<2.5	25
12	Chloramphenicol	<2.5	12.5	25	100

THURSDAY, 6/16/2016

Pick op colonies from Dr. Moreno

- Transformed e. coli w/ lasmids w/ citrine constructs
- Stored at -20 C; marked [LYW 6/16/16]

Talking points to Jaymin in the morning

- the strands
- 10% glycerol (Dr. moreno says it is important to shock the rihzo cells in 10% glycerol)
- 80% glycerol
- sequencing results (usually come at noon)
- assay (set up the citrine assay today)

Dilute over-cultured e.coli w/ entry vector

1:100 dilution: 2970 uL LB + 3 uL 1000x spec + 30 uL over culture
put in 37 C shaking at 12:40, grow until turbid (prob ~3:40)

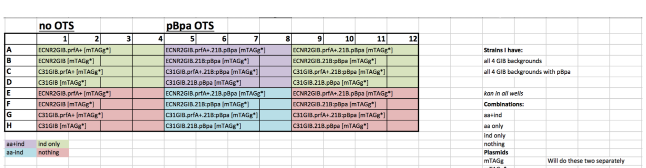
Glycerol Stock

Made 100 mL 80% glycerol [LYW 6/15/16] and 100 mL 10% glycerol [LYW 6/15/16] for future use.
To make the stock sterile: use the vacuum hose in the Isaacs Lab space, suck the mixture through a blueish sand-glass-shape thing labelled [LYW 6/16/16]
***accidentally left the lid open overnight, need to remake the 80% glycerol stock
incubate the cell culture for 3h to grow until turbid (that comes after mid log)
use cryontubes, add 800uL of cell culture and 400 uL of 80% glycerol (Jaymin); 1mL of cell culture and 200 uL of 80% glycerol (Natalie)

96 Well Assay

- Preparation: **3 mL LB w/ 3uL 100x spec grow to mid log OD point** (~3h, put in shaker at 1:40, should be ready at 4:40)
- Chen et al., 2014
  - "For comparison of different promoters, S. meliloti strains were grown in PYE, LB, or M9 liquid media overnight, diluted to the desired optical density, and induced for three hours, to mid-log phase, with 0.2% (13.3 mM) L-arabinose, 0.063% (5 mM) taurine, 0.2% (11.0 mM) L-rhamnose monohydrate, 0.2% (5.6 mM) D-melibiose monohydrate, or 0.2% (3.4 mM) D-raffinose pentahydrate."
  - "the expression system approached maximal induction at lower taurine concentrations in defined medium (15 mM taurine in BM) compared to complex medium (50 mM taurine in RM)"
- Jaymin's suggestion on well design:
  - Make 96 well plates w/ gradient (whatever made sense from the conentration) that go across
  - One roll left emtpy as negative control
  - Measure OD overtime so it has to be in LB
  - once we decide to stop the time course take them out and resuspend in PBS
  - Recipe for making **stock solution for small molecules**: 20% 100mL water 20g of sugar 10/50mL sterilize w/ syringe
  - Recipe for **loading the well**: LB 150 microliters dissolve in water and dilute w/ LB (the process approximately needs 16mL of LB w/ antibiptics in total)
- Natalie's Template for well design

Screen Shot 2016-06-17 at 4.23.56 PM.png



	no OTS				pBpa OTS							
	1	2	3	4	5	6	7	8	9	10	11	12
A	ECNR2GIB.prfA+ [mTAGg*]				ECNR2GIB.prfA+.21B.pBpa [mTAGg*]				ECNR2GIB.prfA+.21B.pBpa [mTAGg*]			
B	ECNR2GIB [mTAGg*]				ECNR2GIB.21B:pBpa [mTAGg*]				ECNR2GIB.21B:pBpa [mTAGg*]			
C	C31GIB.prfA+ [mTAGg*]				C31GIB.prfA+.21B:pBpa [mTAGg*]				C31GIB.prfA+.21B:pBpa [mTAGg*]			
D	C31GIB [mTAGg*]				C31GIB.21B.pBpa [mTAGg*]				C31GIB.21B.pBpa [mTAGg*]			
E	ECNR2GIB.prfA+ [mTAGg*]				ECNR2GIB.prfA+.21B.pBpa [mTAGg*]				ECNR2GIB.prfA+.21B.pBpa [mTAGg*]			
F	ECNR2GIB [mTAGg*]				ECNR2GIB.21B:pBpa [mTAGg*]				ECNR2GIB.21B:pBpa [mTAGg*]			
G	C31GIB.prfA+ [mTAGg*]				C31GIB.prfA+.21B:pBpa [mTAGg*]				C31GIB.prfA+.21B:pBpa [mTAGg*]			
H	C31GIB [mTAGg*]				C31GIB.21B.pBpa [mTAGg*]				C31GIB.21B.pBpa [mTAGg*]			

aa+ind	ind only
aa-ind	nothing

Strains I have:
all 4 GIB backgrounds
all 4 GIB backgrounds with pBpa

kan in all wells
Combinations:
aa+ind
aa only
ind only
nothing
Plasmids
mTAGg Will do these two separately
mTAGg*

- My well design (taurine yet to arrive, characterize the other 6 promoters)

Screen Shot 2016-06-17 at 4.27.22 PM.png

	1	2	3	4	5	6	7	8	9	10	11	12
A	pAStr-CIT N/A pYU3174 EC3056	pAStr-CIT N/A pYU3174 EC3056	pAStr-CIT N/A pYU3174 EC3056	pAStr-CIT N/A pYU3174 EC3056	pAStr-CIT N/A pYU3174 EC3056	pAStr-CIT N/A pYU3174 EC3056	pAStr-CIT N/A pYU3174 EC3056	pAStr-CIT N/A pYU3174 EC3056	pAStr-CIT N/A pYU3174 EC3056	pAStr-CIT N/A pYU3174 EC3056	pAStr-CIT N/A pYU3174 EC3056	pAStr-CIT N/A pYU3174 EC3056
B	pAMed-CIT N/A pYU3166 EC3052	pAMed-CIT N/A pYU3166 EC3052	pAMed-CIT N/A pYU3166 EC3052	pAMed-CIT N/A pYU3166 EC3052	pAMed-CIT N/A pYU3166 EC3052	pAMed-CIT N/A pYU3166 EC3052	pAMed-CIT N/A pYU3166 EC3052	pAMed-CIT N/A pYU3166 EC3052	pAMed-CIT N/A pYU3166 EC3052	pAMed-CIT N/A pYU3166 EC3052	pAMed-CIT N/A pYU3166 EC3052	pAMed-CIT N/A pYU3166 EC3052
C	pAwek-CIT N/A pYU3168 EC3054	pAwek-CIT N/A pYU3168 EC3054	pAwek-CIT N/A pYU3168 EC3054	pAwek-CIT N/A pYU3168 EC3054	pAwek-CIT N/A pYU3168 EC3054	pAwek-CIT N/A pYU3168 EC3054	pAwek-CIT N/A pYU3168 EC3054	pAwek-CIT N/A pYU3168 EC3054	pAwek-CIT N/A pYU3168 EC3054	pAwek-CIT N/A pYU3168 EC3054	pAwek-CIT N/A pYU3168 EC3054	pAwek-CIT N/A pYU3168 EC3054
D	pMel-CIT N/A pYU3163 EC3049	pMel-CIT mel pYU3163 0.02% EC3049 0.56mM	pMel-CIT mel pYU3163 0.04% EC3049 1.12mM	pMel-CIT mel pYU3163 0.06% EC3049 1.68mM	pMel-CIT mel pYU3163 0.1% EC3049 2.8mM	pMel-CIT mel pYU3163 0.15% EC3049 4.2mM	pMel-CIT mel pYU3163 0.2% EC3049 5.6mM	pMel-CIT mel pYU3163 0.4% EC3049 11.2mM	pMel-CIT mel pYU3163 0.6% EC3049 16.8mM	pMel-CIT mel pYU3163 1% EC3049 28mM	pMel-CIT mel pYU3163 1.5% EC3049 42mM	pMel-CIT mel pYU3163 2% EC3049 56mM
E	pRha-CIT N/A pYU3165 EC3051	pRha-CIT rha pYU3165 0.02% EC3051 1.1mM	pRha-CIT rha pYU3165 0.04% EC3051 2.2mM	pRha-CIT rha pYU3165 0.06% EC3051 3.3mM	pRha-CIT rha pYU3165 0.1% EC3051 5.5mM	pRha-CIT rha pYU3165 0.15% EC3051 8.25mM	pRha-CIT rha pYU3165 0.2% EC3051 11.0mM	pRha-CIT rha pYU3165 0.4% EC3051 22mM	pRha-CIT rha pYU3165 0.6% EC3051 33mM	pRha-CIT rha pYU3165 1% EC3051 55mM	pRha-CIT rha pYU3165 1.5% EC3051 82.5mM	pRha-CIT rha pYU3165 2% EC3051 110mM
F	pAra-CIT N/A pYU3167 EC3053	pAra-CIT ara pYU3167 0.02% EC3053 1.33mM	pAra-CIT ara pYU3167 0.04% EC3053 2.66mM	pAra-CIT ara pYU3167 0.06% EC3053 3.99mM	pAra-CIT ara pYU3167 0.1% EC3053 6.65mM	pAra-CIT ara pYU3167 0.15% EC3053 9.975mM	pAra-CIT ara pYU3167 0.2% EC3053 13.3mM	pAra-CIT ara pYU3167 0.4% EC3053 26.6mM	pAra-CIT ara pYU3167 0.6% EC3053 39.9mM	pAra-CIT ara pYU3167 1% EC3053 66..5mM	pAra-CIT ara pYU3167 1.5% EC3053	pAra-CIT ara pYU3167 2% EC3053 133mM
G	pAStr-LIC N/A pYU3155 EC3037	pAMed-LIC N/A pYU3156 EC3038	pAwek-LIC N/A pYU3157 EC3039	pMel-LIC N/A pYU3153 EC3035	pRha-LIC N/A pYU3150 EC3032	pAra-LIC N/A pYU3149 EC3031	pMel-LIC mel pYU3153 0.2% EC3035 5.6mM	pRha-LIC rha pYU3150 0.2% EC3032 11.0mM	pAra-LIC ara pYU3149 0.2% EC3031 13.3mM	LB mel spec 0.2% 5.6mM	LB rha spec 0.2% 11.0mM	LB ara spec 0.2% 13.3mM
H	pAStr-LIC N/A pYU3155 EC3037	pAMed-LIC N/A pYU3156 EC3038	pAwek-LIC N/A pYU3157 EC3039	pMel-LIC mel pYU3153 0.02% EC3035 0.56mM	pRha-LIC rha pYU3150 0.02% EC3032 0.11mM	pAra-LIC ara pYU3149 0.02% EC3031 1.33mM	pMel-LIC mel pYU3153 2% EC3035 56mM	pRha-LIC rha pYU3150 2% EC3032 110mM	pAra-LIC ara pYU3149 2% EC3031 133mM	LB N/A	LB N/A spec	PBS N/A spec

Plating the transformation from yesterday

FRIDAY, 6/17/2016

Meeting w/ Farren

Talking points:

1. Promoter assay: in the plate reader
2. Sequencing results: a few point mutations
3. Suicide vectors: sacB gene, mob region from the broad-hostrange plasmid RP4, may take a week or two
4. PortMage: single colonies for both melilodi and tropicii
5. Experimens w/ Jaymin: tansform MAGE, awaiting results
6. Recombinanses: 7 ready to go, 5 need point mutations, the others unknown

To-dos:

- iGEM group meeting at OML 12:30 pm on Tuesday
- Update the group about analysis on sequencing results
- Email Farren about missing files from Moreno
- *Yale box+attachment: When inform the group about something, attach the file to the email and include a link in the email in Yale box

Sequencing Results for citrine

10-337512224_ab1.zip

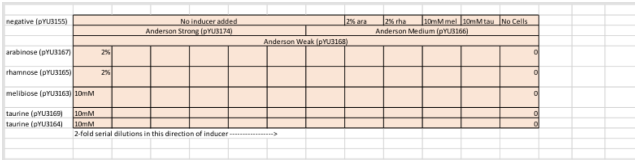
10-337512224_seq.zip

Colin gblocks (2 new, old ones); geneious
Dr. Moreno: get ready for the meeting on Tuesday, figure the g-blocks
Natalie: database for glycerol stocks, make backups for glycerol stocks
Farren: sequecing results; assay results; identify MutS mutL mutH uvrdl; create a strand w/ knockouts each of those; beta suicide vector: design homology
Rhizo antibiotic resistance resullts:???

MONDAY, 6/20

Citrine Fluorescence Assay Data

clipboard_2016-06-21_10:59:07.png



negative (pYU3155)	No inducer added							2% ara	2% rha	10mM mel	10mM tau	No Cells
	Anderson Strong (pYU3174)						Anderson Medium (pYU3166)					
	Anderson Weak (pYU3168)											
arabinose (pYU3167)	2%											0
rhamnose (pYU3165)	2%											0
melibiose (pYU3163)	10mM											0
taurine (pYU3169)	10mM											0
taurine (pYU3164)	10mM											0
	2-fold serial dilutions in this direction of inducer ----------------->											

2uL mid-log cells
are innoculated in 150uL media (all wells contain 100ug/mL spectinomycin) in blackwell 96 well plate. OD600 and Citrine (505nm excitation and 535nm emission) are measured over 16 hours. At the final timepoint, cells are spun down and resuspended in PBS and measured.

Table7

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1		1	2	3	4	5	6	7	8	9	10	11	12	
2	A	260	238	263	271	238	258	289	213	293	255	260	178	Citrine:505,535
3		0.685	0.647	0.596	0.629	0.529	0.572	0.553	0.488	0.8	0.704	0.606	0.045	OD:600
4	B	296	297	324	313	330	311	411	425	388	390	429	370	Citrine:505,535
5		0.692	0.785	0.793	0.805	0.802	0.84	0.848	0.832	0.847	0.917	0.849	0.763	OD:600
6	C	403	393	471	432	516	424	432	425	445	402	399	467	Citrine:505,535
7		0.706	0.706	0.857	0.756	0.861	0.701	0.787	0.701	0.673	0.63	0.641	0.728	OD:600
8	D	261	313	359	336	371	438	366	332	493	462	467	415	Citrine:505,535
9		0.599	0.627	0.704	0.633	0.661	0.728	0.664	0.637	0.928	0.881	0.862	0.718	OD:600
10	E	4404	3127	8711	4559	3472	3019	3250	2758	2965	2806	2643	2734	Citrine:505,535
11		0.907	0.938	1.018	0.768	0.707	0.561	0.602	0.541	0.562	0.529	0.511	0.552	OD:600
12	F	317	245	266	253	274	282	280	285	287	294	277	291	Citrine:505,535
13		0.902	0.716	0.672	0.626	0.645	0.651	0.638	0.629	0.644	0.659	0.694	0.696	OD:600
14	G	473	561	566	539	491	463	451	413	422	423	446	361	Citrine:505,535
15		0.646	0.731	0.654	0.64	0.679	0.575	0.583	0.623	0.606	0.621	0.66	0.572	OD:600
16	H	347	349	380	391	419	399	401	434	460	422	409	429	Citrine:505,535
17		0.473	0.474	0.533	0.584	0.595	0.588	0.607	0.595	0.683	0.623	0.537	0.517	OD:600

Jaymin's interpretation for the Citrine Fluorescence Assay Data
Citrine expression is only seen in rhamnose construct, where it is largely constitutive.
Issues:

- Anderson promoters have a poor ribosome binding site
- Rhamnose promoter is controlled by a DeoR-like repressor not found in E coli (and therefore expression is constitutive)
- Arabinose promoter is controlled by a lysR-family activator not found in E coli (and therefore expression is inactive)
- Melibiose promoter is controlled by a araC-family activator not found in E coli (and therefore expression is inactive)
- Taurine promoter is controlled by a MocR-family activator not found in E coli (and therefore expression is inactive)

The needed regulatory proteins are natively in Meliloti, and so these plasmids should function more readily in that organism

Knocking out MutS in rhizo

- suicide vector
  - cos lambda phage site allows for insertion of 45 kb sequences
  - lethal gene eg SacB causes death on sucrose 5%
- homologous proteins in rhizo
  - MutS mutL mutH uvrd
  - use biocyc to find the sequences: http://biocyc.org/

TUESDAY, 6/21

Logistics

- [ ] talk to the iGEM person and become an liason
- [ ] Talk to John the West campus guy
- [ ] Grant Application: http://2016.igem.org/Sponsors/Syngenta **July 1st**
- [ ] Team Overview: **July 1st**
- [ ] Safety form: July 15th

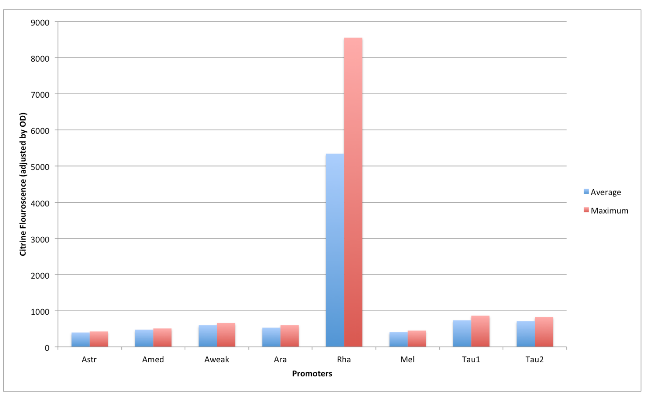
OneNote link

https://onedrive.live.com/redir?page=view&resid=5B2FA8C3D5BC675D!129&authkey=!AB5BmlWVeN8PGyo

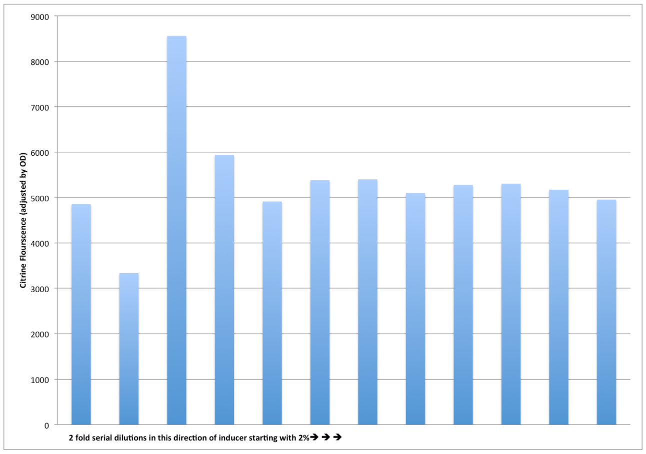
Figures for Citrine Fluorescence Assay Data

20160621 Data Interpretation for Citrine Fluorescence Assay Data.xlsx

Promoters.png



Rha.png



Do what Jaymin recommended
ID: 16s seuqencing

Meeting w/ PIs at 12:30pm

- NCBI Primer BLAST: to see if primer has identical sequence within the host genome
- Long term concern: we might need to make comp cells every single time to do MAGE, a more efficient way
- Bookkeeping tips: table, the different samples, whether or not DNA was added, the number of clones for each
- Rzhio
  - check the literature for ciat rif resistance
  - rhizo comp cells can be stored for future eporation
- growth/spec resistance: the strains may be switched (Jaymin is sequencing)
- nodulation: cowpea = most sensitive for nodulation assays
  - CIAT spp nodulate trees, beans, generally more finicky
  - meliloti
- Sequencing reactions for citrine: terminators have a lot of structure, for the terminator sequences; mfold for secondary structure on the terminator
- Consituitive promoters
  - RBS is really bad & at the wrong place
  - FIX the rbs problem: lic in the correct RBS for constitutive
  - SacB has its own independent promoter within
- Next step for pORTMAGE3: knock out mutS with pORTMAGE3 once transformed into Rhizo

WEDNESDAY, 6/22

Transformation of Citrine Constructs in Rhizo

IMG_2657.JPG

Electroporation of Plasmid DNA into Electrocompetent Rhizobium Cells

*no pipetting up & down
mix by tapping
briefly ~~con~~centrifuging

S: meliloti
R: tropicii

1. Obtain an aliquot of electro-competent rhizobium cells for the -80 °C freezer. 10^{10} CFU
2. Thaw the cells on ice. [plasmid] × 25 ng/mL dilution w/ water → 20 ng/mL concentration for DNA solution
3. Add the transforming DNA to the cells. Aim for a concentration range between 10-20 ng per transformation experiment. only pipet 1uL into the cells (not dilute them)
   Note: It is important to not add more than 2 ul of liquid to the transformation mixture. It is also critical that the DNA solution is very low in salts. This will prevent arching of the samples during the electroporation process. 1mL of working solution → stab & spin
4. Mix the DNA cell mixture gently by tapping the cell tube. If necessary, briefly centrifuge the mixture to make sure all the contents are at the bottom of the tube.
5. Carefully transfer by pipetting 20 µL the DNA-cell mixture to the electroporation cuvette. Be sure to tap the cuvette against the bench to ensure that the cells are evenly distributed in the bottom of the cuvette. The should be no air bubbles. Place the cuvette on ice while you set up the electroporation instrument (BioRad Gene Pulser)
6. Set up the BioRad Gene Pulser as follows for 0.2cm ~~2 mL~~ gap *if the gap is smaller, cut the conditions by half
   Volts = 2.5 KV (use the Set Volts button)
   Capacitance = 250 uF (↑)
   Resistance = 200 Ohms (↓) $1 cm = 10^{-2} m$ $1mm = 10^{-3} m$
   Note: These settings are *specific* for a ~~2mm~~ 0.2cm Electroporation cuvette.

*no second push of pipetting
tap the cuvette before putting into the chamber

7. Wipe the cuvette to make sure the outside is dry. Place the cuvette in the chamber, and set the chamber all the way back such that the cuvette is in contact with the electrodes.
8. Press the Pulse buttons simultaneously until you hear the "beep" sound. The voltage display will flicker as the samples are being electroporated.

IMG_2658.JPG

Once you have heard the "beep", press the Time Constant Button and record the number being displayed.

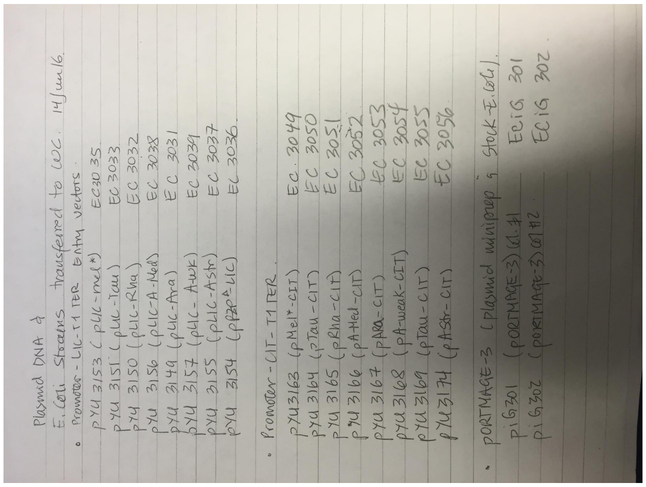
9. Once the electroporation has been completed, add 1 ml of LB to the cell-DNA mixture.
10. Carefully transfer the entire solution to a culture tube by pipetting. We use 15 ml falcon tubes (Ref. 352059)
11. incubate the cultures with aeration (shaking) for 4 hours at 30 °C. permisive temperature 28-30°C
12. Plate the cells on LB medium supplemented with the appropriate antibiotic for selection. 100 mg/uL spec
    Note: since we are transforming whole plasmids as opposed to ligation or annealing reactions, the transformation efficiency will be very high. Plate different dilutions to ensure that we can obtain samples where we can pick single colonies to validate.

different dilutions 10/20 ~~mg~~ mL of direct transformation solution
additional LB: enough liquid for nice dispertion for the beads
100 mL

Reference for pYU numbers for constructs

4 promoters used in transformation: pYU

IMG_2637.JPG



To-do list at WC

- Find my mac charger
- Make 3 sleeves of plates w/ spec
- Drop off Mindy's pipets
- Pick up the lab notebook for Mindy

Plating of the cultrued rhizo w/ citrine w/ inducible promoters

After incubating while shaking at 30C for 4h, each solution was plated on 3 spec plates. (24 in total)

Table8

	A	B	C	D
1		Plate 1	Plate 2	Plate 3
2	Culture added	10 uL	20 uL	50 uL
3	LB added	100 uL	100 uL	100 uL

Labeling example [100ng/mL spec_pYU3163_R_10uL+100uL LB LYW 6.22.16]

Placed in 28C incubator, need to grow for 2-3 days

THURSDAY, 6/23

Colin found gBlocks for the recombinases from last year. Jinny designed some primers for point mutagensis and brought working solutions of the gBlocks to OML.

FRIDAY, 6/24

Adding new RBS to citrine for the Anderson promoters

some useful websites:

http://denovodna.com/ - design RBS for known CDS, a score out of 100, 000, usually 1-10,000 is good enough

http://unafold.rna.albany.edu/ - folding structures for RNA/DNA/protein

http://arep.med.harvard.edu/kzhang/cgi-bin/myOligoTm.cgi - calculate annealing temperature for primer design

Jaymin ordered primer for amplification of RBS_citrine. Added to the primer library as

Some inducers

Brought stock solutions of inducers to OML

(Issacs Lab space have Rha and ARa, iGEM space has the tuarine)

Ordered Mel molecules through Amy

- double check when it arrives

No growth for the Transformation of Citrine Constructs in Rhizo

MONDAY, 6/27

[Constitutive Promoters]

No growth for the Transformation of Citrine Constructs in Rhizo

Found contamination on some plates.

The plates were marked purple, which was not found in the color-coding system>>may not be spec plates after all.

Re-do Transformation of Citrine Constructs in Rhizo

Same protocal as Wed 6.22.16.

- Use 18 culture tubes this time w/ different DNA concentration.
- Used 2 batches of comp cells from different dates.
- 16 tubes for (4 promoters*2 strains*2 concentrations)

Plating: After incubating while shaking at 30C for 4h [somebody stopped the incubator halfway], each solution was plated on 3 spec plates. (54 in total)

Table9

	A	B	C	D
1		Plate 1	Plate 2	Plate 3
2	Culture added	10 uL	20 uL	80 uL
3	LB added	90 uL	80 uL	20 uL

Meeting w/ Prof. Dellaporta

- Upload the slides
- Contamination issues

TUESDAY, 6/28

[Constitutive Promoters]

RBS_citrine Primer was resynthesized

Colin will bring to meeting tmr at 10:30, lobby of KBT

[Inducible Promoters]

Plating of Citrine Constructs in Rhizo

The plates were newly made, left on the bench overnight to dry. Placed in the 28 C incubator at 11:00 am.

WEDNESDAY, 6/29

[Inducible Promoters]

Plating of Citrine Constructs in Rhizo

Growth found on some plates.

Consistenely on 20+80 diluted for S and 20+80 stock for R. S colonies similar to e.coli, R colonies relatively smaller.

Will perform selection on plates and colony PCR tomorrow.

Not sure of colony PCR conditions, will do a test run (2DMSO*2MM*2Strains*2 neg con)

10x MM made and stored in -20 C for both +/- DMSO

[Constitutive Promoters]

PCR Amplification of RBS_citrine

Protocol:

IMG_2705.JPG

General PCR Protocol

PCR amplification:

Before beginning, make sure the contents of the tubes are thawed completely, mixed and spun briefly in the tabletop microfuge.

Note: a new clean tip must be used for every step in the protocol. Never reuse tips!!!! The PCR reaction should be set up on ice. The first tube containing 12.5 µl of 2X Phusion Master Mix (Phusion High-Fidelity DNA Polymerase, nucleotides and optimized reaction buffer including $MgCl_2$) will be provided to you. You will need to add: water, primers and template. The final volume of the reaction will be 25 µl.

1. Assembly of PCR Reactions:

Component	Volume	Final Concentration
2X Phusion Master Mix	12.5 µl	1X
dH_2O	X µl	
Forward Primer (10 µM)	1.3 µl	0.52 µM
Reverse Primer (10 µM)	1.3 µl	0.52 µM
Template		
Genomic DNA (50 ng/µl)	1.0 µl	2 ng/µl
DMSO (100%)	0.5 µl	2 %

2. General Cycling Instructions:

Cycle Step	Temperature	Time	Number of Cycles
Initial Denaturation	98° C	30 seconds	1
Denaturation	98° C	10 seconds	30
Annealing	42° C — 65° C	30 seconds	
Extension	72° C	1min/kb	
Final Extension	72° C	5 minutes	1
	4° C	Hold	

Leave your PCR reaction on ice and wait until all students are ready to proceed to the cycling steps. A program file has already been set up for you. Once the PCR reaction is completed, the tubes will be removed from the machine and stored at 4° C until the next lab.

MCDB 201Lb S'16 2

Screen Shot 2016-06-30 at 12.55.57 PM.png

PCR Set-up for Amplifying Citrine with New RBS /uL

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
		HF								GC							
		RBS				C+				RBS				C+			
		+DMSO		-DMSO		+DMSO		-DMSO		+DMSO		-DMSO		+DMSO		-DMSO	
		Template	C-	Template	C-	Template	C-	Template	C-	Template	C-	Template	C-	Template	C-	Template	C-
HF	Master Mix	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
GC	Master Mix	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
priG 397	F-RBS 10uM	1.3	1.3	1.3	1.3	0.0	0.0	0.0	0.0	1.3	1.3	1.3	1.3	0.0	0.0	0.0	0.0
p3743	F-pos con 10	0.0	0.0	0.0	0.0	1.3	1.3	1.3	1.3	0.0	0.0	0.0	0.0	1.3	1.3	1.3	1.3
p3745	R-universal 1	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
DMSO		0.5	0.5	0.0	0.0	0.5	0.5	0.0	0.0	0.5	0.5	0.0	0.0	0.5	0.5	0.0	0.0
pYU3034-2	template 5ul	1.0	0.0	1.0	0.0	1.0	0.0	1.0	0.0	1.0	0.0	1.0	0.0	1.0	0.0	1.0	0.0
dH2O		8.4	9.4	8.9	9.9	8.4	9.4	8.9	9.9	5.9	6.9	6.4	7.4	5.9	6.9	6.4	7.4
Total		25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0

Master Mix Design /uL

		RBS					C+				
		priG 397	p3745	DMSO	dH2O	Total	p3743	p3745	DMSO	dH2O	Total
+DMSO	1x	1.3	1.3	0.5	8.4	11.5	1.3	1.3	0.5	8.4	11.5
	5x	6.5	6.5	2.5	42.0	57.5	6.5	6.5	2.5	42.0	57.5
-DMSO	1x	1.3	1.3	0.0	8.9	11.5	1.3	1.3	0.0	8.9	11.5
	5x	6.5	6.5	0.0	44.5	57.5	6.5	6.5	0.0	44.5	57.5

Add MM to Tubes

	RBS	C+
+DMSO	01,02,09,10	05,06,13,14
-DMSO	03,04,11,12	07,08,15,16

Add Template to Tubes

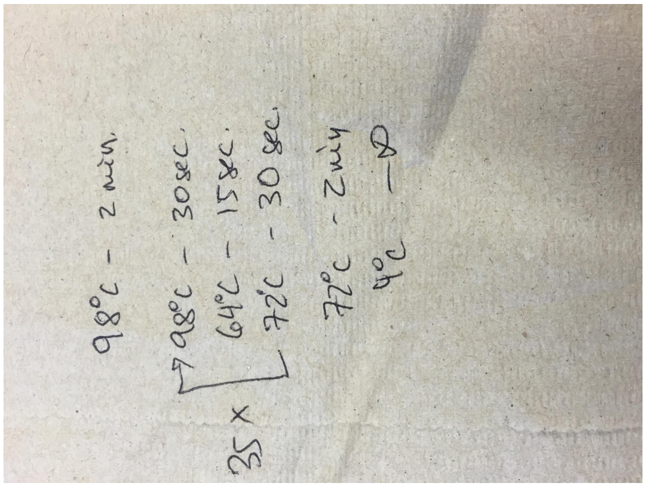
Template	01,03,05,07,09,11,13,15
C-	02,04,06,08,10,12,14,16

AGE Recipe /uL

10x Loading	PCR Product
5.0	8.0
Ladder	
5.0	

PCR conditions:

IMG_2704.JPG



AGE (8uL sample+ 3uL 10x loading dye against 1kb ladder, 110V for 30min)

Leslie 6.29.16 PCR amp of RBS_citrine.pdf

Meeting with Colin and Jaymin in KBT, logistics

- Set-up a meeting w/ Colin next week on Biobricks
- Safety forms
- Email people about polymerases, self dimeratization with RBS
- [x] Email Jaymin and Colin about the shinero
    - http://2012.igem.org/Team:Minnesota/Project/UV_Absorption
    - http://2015.igem.org/Team:SF_Bay_Area_DIYBio/Description
    - https://www.addgene.org/vector-database/2569/
    - http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3116657/

THURSDAY, 6/30

[Inducible Promoters]

Incubation results for plating with gradient

- S. meliloti:
    - clear colonies on "diluted 10+90" "diluted 20+80",
    - reduced colonies on "stock 10+90" "stock 20+80" "diluted 75+25",
    - too much colonies on "stock 75+25"
- R. tropicii:
    - clear colonies on "diluted 10+90"
    - too much colonies on "diluted 20+80" "stock 10+90" "stock 20+80" "diluted 75+25" "stock 75+25"

Colony PCR for Rhzio with citrine - Test Run for MM and DMSO

Choose pYU3167 diluted 10+90 for both R strain and S strain

Add 12.5 uL of +/- DMSO to HF/GC MM (2 strains* 2DMSO* 2 MM)

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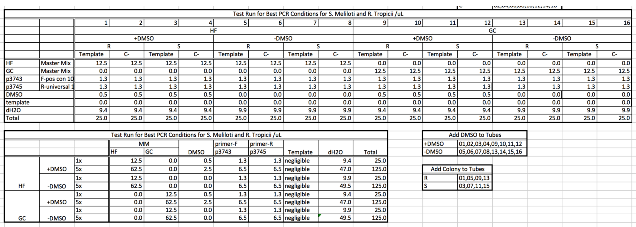


Test Run for Best PCR Conditions for S. Meliloti and R. Tropicii /uL																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
		HF								GC							
		+DMSO				-DMSO				+DMSO				-DMSO			
		R		S		R		S		R		S		R		S	
		Template	C-	Template	C-	Template	C-	Template	C-	Template	C-	Template	C-	Template	C-	Template	C-
HF	Master Mix	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
GC	Master Mix	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
p3743	F-pos con 10	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
p3745	R-universal 1	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
DMSO		0.5	0.5	0.5	0.5	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.5	0.0	0.0	0.0	0.0
template		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
dH2O		9.4	9.4	9.4	9.4	9.9	9.9	9.9	9.9	9.4	9.4	9.4	9.4	9.9	9.9	9.9	9.9
Total		25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0

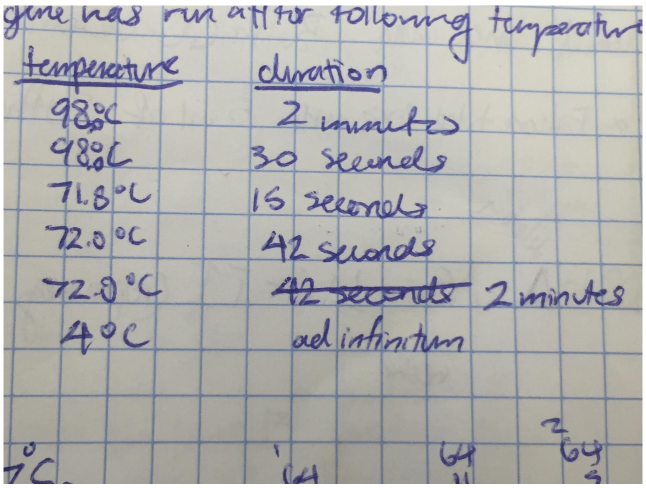
Test Run for Best PCR Conditions for S. Meliloti and R. Tropicii /uL										
			MM		DMSO	primer-F	primer-R	Template	dH2O	Total
			HF	GC		p3743	p3745			
HF	+DMSO	1x	12.5	0.0	0.5	1.3	1.3	negligible	9.4	25.0
		5x	62.5	0.0	2.5	6.5	6.5	negligible	47.0	125.0
	-DMSO	1x	12.5	0.0	0.0	1.3	1.3	negligible	9.9	25.0
		5x	62.5	0.0	0.0	6.5	6.5	negligible	49.5	125.0
GC	+DMSO	1x	0.0	12.5	0.5	1.3	1.3	negligible	9.4	25.0
		5x	0.0	62.5	2.5	6.5	6.5	negligible	47.0	125.0
	-DMSO	1x	0.0	12.5	0.0	1.3	1.3	negligible	9.9	25.0
		5x	0.0	62.5	0.0	6.5	6.5	negligible	49.5	125.0

Add DMSO to Tubes	
+DMSO	01,02,03,04,09,10,11,12
-DMSO	05,06,07,08,13,14,15,16

Add Colony to Tubes	
R	01,05,09,13
S	03,07,11,15

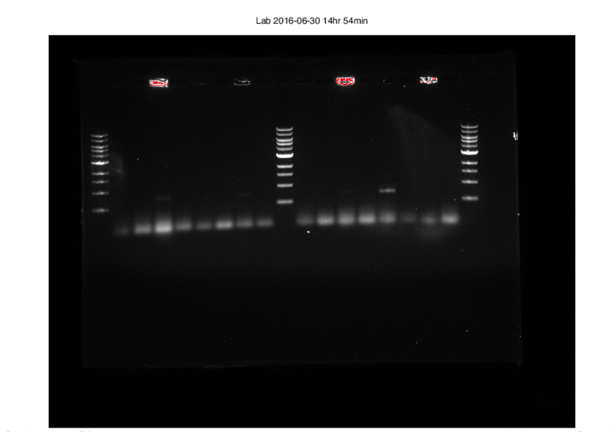
PCR conditions
same as citrine amplification in MCDB 201L

FullSizeRender 2.jpg



AGE result (8uL sample+ 4uL 10x loading dye against 1kb ladder, 110V for 30min)

Leslie 6.30.16 Test run colony PCR.pdf
p. 1



Dr. Moreno's commets: replace the 98C for 2min at the beginning of the PCR with 95C for 5min so that all plasmids within the strains can denature properly.

Actual Run - Colony PCR for Rhzio with citrine
AGE results show GC w/o DMSO was the best PCR condition for both strains.

Screen Shot 2016-07-01 at 10.20.33 AM.png

Colony PCR for Screening Recombinant Rhizo Cells with Inducible Promoter_Citrine Constructs /uL								
	R				S			
	pYU 3143	pYU 3147	pYU 3145	pYU 3144	pYU 3143	pYU 3144	pYU 3145	pYU 3147
A	1	9	17	25	33	41	49	57
B	2	10	18	26	34	42	50	58
C	3	11	19	27	35	43	51	59
D	4	12	20	28	36	44	52	60
E	5	13	21	29	37	45	53	61
F	6	14	22	30	38	46	54	62
G	7	15	23	31	39	47	55	63
neg con	8	16	24	32	40	48	56	64

Colony PCR for Screening Recombinant Rhizo Cells with Inducible Promoter_Citrine Constructs /uL							
	GC	DMSO	p3743	p3745	Template	dH2O	Total
1x	12.5	0.0	1.3	1.3	negligible	9.9	25.0
70x (64 reac	875	0	91	91	negligible	693	1750

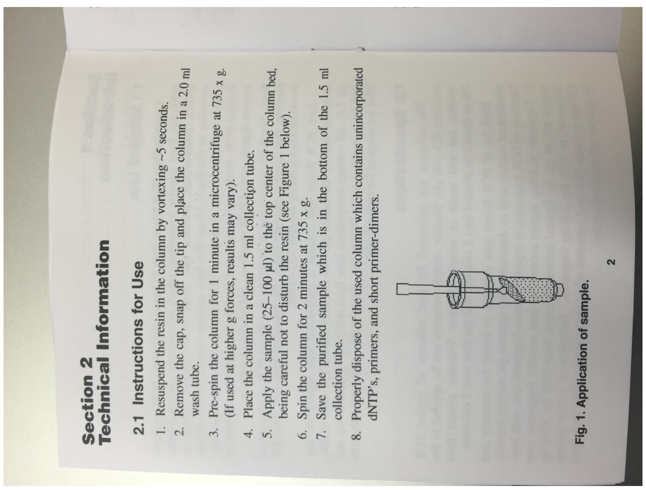
Same PCR Conditions as the test run.
Plates (4, half for each construct) stored at 28C for incubation.

[Constitutive Promoters]

Purification of PCR results of RBS-citrine amplification

Protocol:

IMG_2698.JPG



Section 2
Technical Information

2.1 Instructions for Use

1. Resuspend the resin in the column by vortexing ~5 seconds.
2. Remove the cap, snap off the tip and place the column in a 2.0 ml wash tube.
3. Pre-spin the column for 1 minute in a microcentrifuge at 735 x g. (If used at higher g forces, results may vary).
4. Place the column in a clean 1.5 ml collection tube.
5. Apply the sample (25–100 µl) to the top center of the column bed, being careful not to disturb the resin (see Figure 1 below).
6. Spin the column for 2 minutes at 735 x g.
7. Save the purified sample which is in the bottom of the 1.5 ml collection tube.
8. Properly dispose of the used column which contains unincorporated dNTP's, primers, and short primer-dimers.

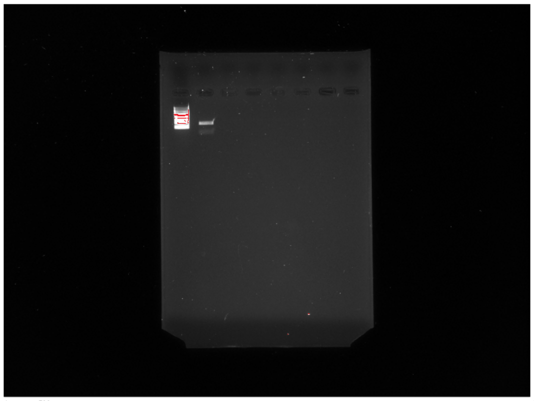
Fig. 1. Application of sample.

2

15 uL of reaction 9 and reaction 11 were added to the column to spin down, respectively.
Purified results:
AGE (4uL sample+ 4uL 10x loading dye against 1kb ladder, 110V for 15min)

Leslie 6.30.16 Purification of RBS_citrine.pdf
p. 1

Lab 2016-06-30 12hr 26min



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Nanodrop (add 2uL sample)

FullSizeRender 3.jpg

T4 Treatment of Insert (RBS_citrine)
Protocol:

Screen Shot 2016-06-30 at 12.52.31 PM.png

PCR Insert-T4DNA Polymerase Treatment:

1. In a clean, sterile 0.2 ml PCR tube, mix the following components:

Reagent	*Volume (microliters-µl)*
Column-purified PCR insert	5.0 µl
dH2O	1.5 µl
10X T4 buffer (NEB 2)	1.0 µl
dCTP @ 25 mM	1.0 µl
1M DTT	0.5 µl
100 X BSA (NEB)	0.5 µl
T4 DNA polymerase	0.5 µl
Final Reaction Volume	**10.0 µl**

2. Incubate the mixture at 22°C for 30 minutes in a thermocycler.

3. Incubate the mixture at 70°C for 20 minutes in a thermocycler. This step will heat inactivate the T4 DNA Polymerase.

Made double the volume listed in the protocol

Ligation Indepedent Reaction

Protocol:

Screen Shot 2016-06-30 at 3.10.20 PM.png

1. In a 1.5 ml eppendorf tube, mix the following:

Reagent	Volume (microliters-µl)
BsaI-T4 treated vector	1 µl
T4 treated PCR insert	2 µL

Note: If you experience trouble pipetting such small volumes accurately, set a 2X reaction, where the final volume will be 6 µL.

2. Incubate 10 minutes at 22°C. Place on ice.
   *Note: at this point, the LIC reaction is ready to be used for **Bacteria Transformation** in order to recover the recombinant plasmid molecule.*

   IMPORTANT: You may proceed to the next step, or store your annealed DNA complex at 4°C for later use.

Double the volume when piptetting for higher accuracy.

3 vector backbones: pYU 3155, pYU 3156, pYU 3157

Table10

	A	B	C	D
1	308	pYU3155 (pA-Str-LIC-T1Ter)	MCDB201Lb	pPZP200b-Del
2	309	pYU3156 (pA-Med-LIC-T1Ter)	MCDB201Lb	pPZP200b-Del
3	310	pYU3157 (pA-wk-LIC-T1Ter)	MCDB201Lb	pPZP200b-Del

E. coli transformation of plasmids w/ RBS_c

Screen Shot 2016-06-30 at 3.11.57 PM.png

1. To the "annealed DNA complex" from **step IV**, add 50 µl of competent cells (Invitrogen TOP 10).

   Note: Be careful to pipette the cells gently without introducing bubbles to the DNA mix. You may mix the contents of the tube by gently tapping it with your fingers, and quickly bring the contents of the tube to the bottom by briefly spinning the tubes in the Nanofuge.

2. Incubate on ice for 15 minutes
3. Briefly heat-shock the mixture by incubating at 42°C for 30 seconds.
4. Quickly place on ice for 2 minutes.
5. Add 100 µl of SOC medium. Incubate the transformed cells at 37°C, shaking for 1 hour.
6. Plate 100µl cells on an LB plate containing the appropriate antibiotic and 5% sucrose. For detailed instructions on how to plate bacteria, see the **Spreading Bacteria with Glass Beads** protocol.

3 tubes of competent cells obtained from Yingchun, added 150 uL SOC to each, incubated shaking at 37 C for an hour.

Centrifudge for 1 min, disposed 150 uL supernatant, plated the remaining 150 uL w/ beads. Kept at 37 C for incubation.

Labelled as **[LYW 6.30.16 e.coli top10 pYU 3155/6/7 RBS_citrine]**

FRIDAY, 7/1

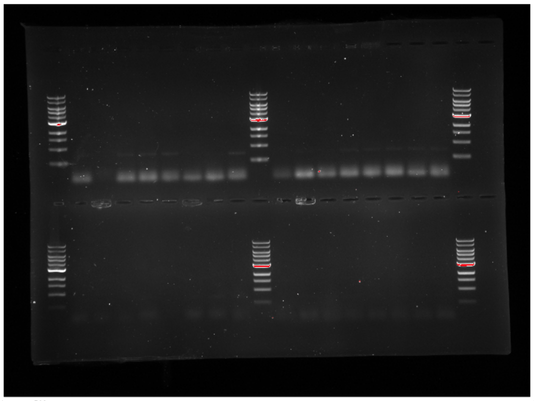
[Inducible Promoters]

AGE of Colony PCR for Screening Recombinant Rhizo Cells with Inducible Promoter_Citrine Constructs

(8uL sample+ 4uL 10x loading dye against 1kb ladder, 110V for 30min)

[Original] Rhzio w Citrine Colony PCR-1.pdf
p. 1

Lab 2016-07-01 12hr 24min



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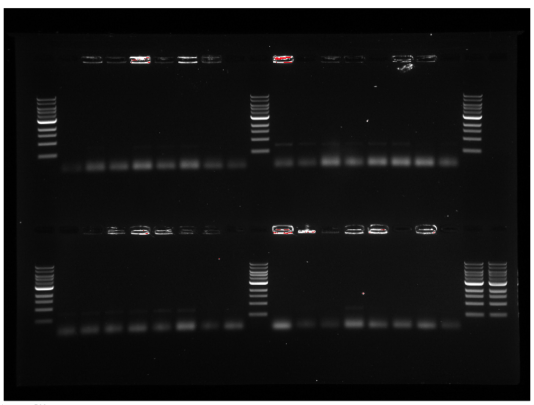
[Original] Rhzio w Citrine Colony PCR-2.pdf
p. 1

Lab 2016-07-01 12hr 14min



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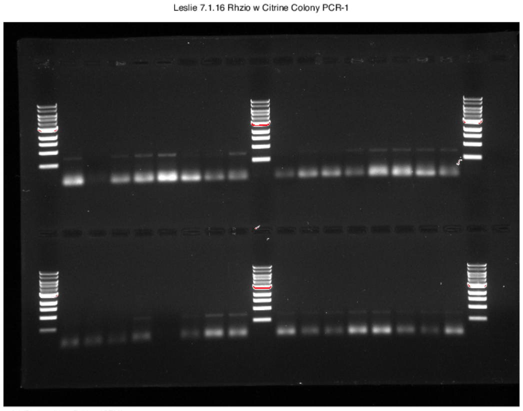
The bands were fuzzy at 800 bp. Repeat AGE with (17uL sample+ 4uL 10x loading dye against 1kb ladder, 110V for 30min)

[Repeated] Rhzio w Citrine Colony PCR-1.pdf
p. 1

Leslie 7.1.16 Rhzio w Citrine Colony PCR-1



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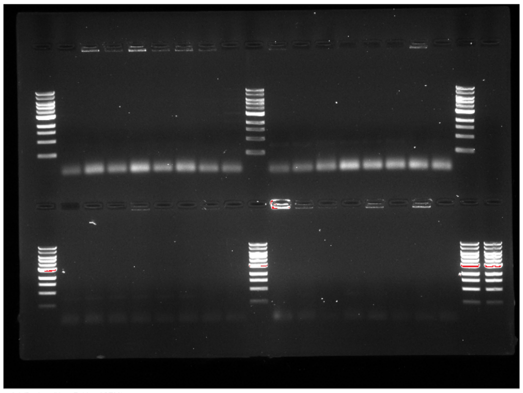
[Repeated] Rhzio w Citrine Colony PCR-2.pdf
p. 1

Leslie 7.1.16 Rhzio w Citrine Colony PCR-2



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Printed: 7/1/16 2:21 PM
Page 1 of 1

[Constitutive Promoters]

Good colonies found on all 3 plates: [LYW 6.30.16 e.coli top10 pYU 3155/6/7 RBS_citrine]
Colonies PCR Setup

Screen Shot 2016-07-01 at 2.56.19 PM.png

Colony PCR for Screening Recombinant E. coli Cells with RBS_Citrine Constrcuts /uL								
	A	B	C	D	E	F	G	neg con
pYU 3155	1	2	3	4	5	6	7	8
pYU 3156	9	10	11	12	13	14	15	16
pYU 3157	17	18	19	20	21	22	23	24

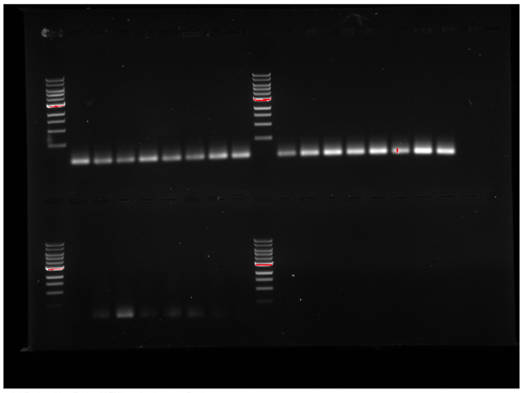
Colony PCR for Screening Recombinant Rhizo Cells with Inducible Promoter_Citrine Constructs /uL							
	GC	DMSO	prig397	p3745	Template	dH2O	Total
1x	12.5	0.0	1.3	1.3	negligible	9.9	25.0
30x (24 react	375	0	39	39	negligible	297	750

]

Plates: did striking on spec, should have done with both spec and spec+sur, Yingchun will do the striking on Sunday
AGE (8uL sample+ 4uL 10x loading dye against 1kb ladder, 110V for 30min)

new RBS.pdf
p. 1

new RBS



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Page 1 of 1

No clear band was found anywhere. Yingchun will do the single colony striking on spec+suc over the weenend, waiting on those resukts.

TUESDAY, 7/5

Emails

- Email Colin + Jaymin about application.
- Emailed Mindy about project description.
- [ ] Email Gloria and Mindy about self-introduction.
- [x] Set-up a meeting w/ Colin next week on Biobricks
- [ ] Safety forms
- [ ] Email people about polymerases, self dimeratization with RBS
- [ ] Find Mel on west campus

Pepper: website for find procarykotic promoters
http://server.molgenrug.nl/index.php/prokaryote-promoters
Fisher Scientific: primer dimer check

[Constitutive Promoters]
Good colonies found on all 3 plates spec+suc: [LYW 7.1.16 e.coli top10 pYU 3155/6/7 RBS_citrine]
Repeat Colony PCR

Screen Shot 2016-07-01 at 2.56.19 PM.png

Colony PCR for Screening Recombinant E. coli Cells with RBS_Citrine Constrcuts /uL								
	A	B	C	D	E	F	G	neg con
pYU 3155	1	2	3	4	5	6	7	8
pYU 3156	9	10	11	12	13	14	15	16
pYU 3157	17	18	19	20	21	22	23	24

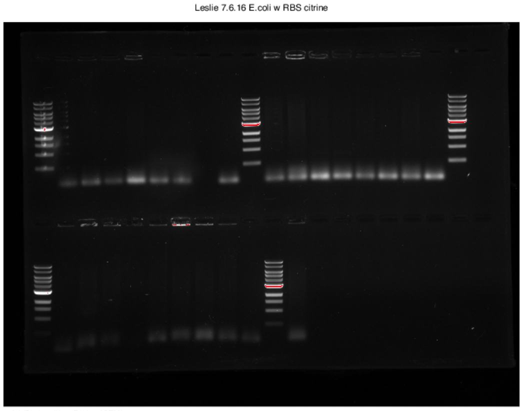
Colony PCR for Screening Recombinant Rhizo Cells with Inducible Promoter_Citrine Constructs /uL							
	GC	DMSO	prig397	p3745	Template	dH2O	Total
1x	12.5	0.0	1.3	1.3	negligible	9.9	25.0
30x (24 react	375	0	39	39	negligible	297	750

]

PCR conditions:
(1x) 98C 5min (35x) 98C 30s, 64C 15s, 72C 30s (1x) 72C 5min, 4C forever

Leslie 7.6.16 E.coli w RBS citrine.pdf
p. 1

Leslie 7.6.16 E.coli w RBS citrine



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Page 1 of 1

[Inducible Promoters]
Analyzed results from colony PCR and plates.
For R. tropicii, identfied one colony to strike on each construct.
Striking the

For S. meliloti, contamination found on both plates and colony PCR results almost nonexsitent.

Repeat Colony PCR (used the wrong primers!!! should be p3743 and p3745 instead of p3143 and p3145)
Conditions: (1x) 98C for 5min (35x) 98C for 30s, 65C for 15s, 72C for 30s (1x) 72C for 2min, 4C forever

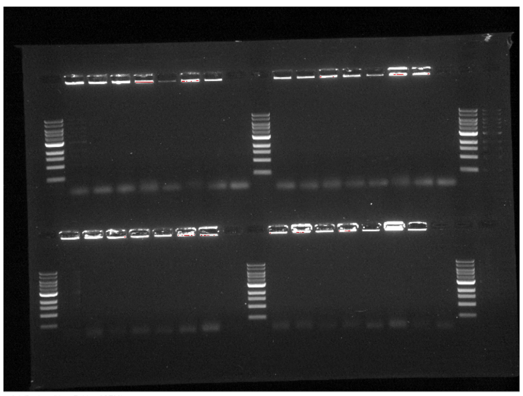
Screen Shot 2016-07-06 at 11.46.02 AM.png

Colony PCR for Screening Recombinant Rhizo Cells with Inducible Promoter_Citrine Constructs /uL								
	S				R			
	pYU 3143	pYU 3144	pYU 3145	pYU 3147	pYU 3143	pYU 3144	pYU 3145	pYU 3147
A	1	9	17	25	33	41	49	57
B	2	10	18	26	34	42	50	58
C	3	11	19	27	35	43	51	59
D	4	12	20	28	36	44	52	60
E	5	13	21	29	37	45	53	61
F	6	14	22	30	38	46	54	62
G	7	15	23	31	39	47	55	63
neg con	8	16	24	32	40	48	56	64

Colony PCR for Screening Recombinant Rhizo Cells with Inducible Promoter_Citrine Constructs /uL							
	GC	DMSO	p3143	p3145	Template	dH2O	Total
1x	12.5	0.0	1.3	1.3	negligible	9.9	25.0
70x (64 react	875	0	91	91	negligible	693	1750

Leslie 7.6.16 Rhizo w Citrine.pdf
p. 1

Leslie 7.6.16 Rhizo w Citrine



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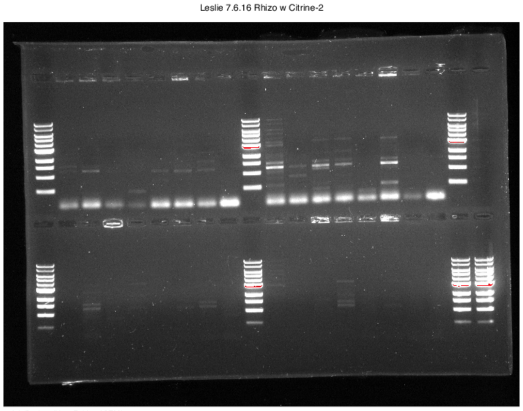
Page 1 of 1

Leslie 7.6.16 Rhizo w Citrine-2.pdf
p. 1

Leslie 7.6.16 Rhizo w Citrine-2



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Page 1 of 1

Knocking out mutS with bla (ampicillin/carbenicillin resistance protein)

- CIAT:
  - R-mut-bla-f: CGGCGCGCGG ATAGGGTGGC ACCTCATTTT TTTGGAAAAA CTGCACCTCCGGAGCGGATTTGAACGTTGCG

  - R-mut-bla-r: CGAGAAGAACTTCGAGATACATCATATTGCCCTTGGAATATCGCCTGTGGTTACCAATGCTTAATCAGTGAGGC
  - R-mut-seq-f: ATAGCTGTTGTCTTCTATAGCGG
  - R-mut-seq-r: ACCCAGAATGCGGACTTC
- Meliloti:
  - S-mut-bla-f: CGTCTATAAATGCTTGCGGATAGTGTGACATCTCTTTTTTTGGAACAATCGGAGCGGATTTGAACGTTGCG
  - S-mut-bla-r: CTGTATAGCCCTTTTGCCGGCCGAATGACAGGGGAAATAGCCGGGCGAAATTACCAATGCTTAATCAGTGAGGC
  - S-mut-seq-f: GTCAGCCTCTTTGTTGTTGTC
  - S-mut-seq-r: ATCATTCGTTGCAAGCGC
- Amplify ampR from pBAD-hisB:
GGAGCGGATTTGAACGTTGCGAAGCAACGGCCCGGAGGGTGGCGGGCAGGACGCCCGCCATAAACTGCCAGGCATCAAATTAAGCA
GAAGGCCATCCTGACGGATGGCCTTTTTGCGTTTCTACAAACTCTTTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAG
ACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGC
GGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTAC
ATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCT
ATGTGGCGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACT
CACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGC
CAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGT
TGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACT
ATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGC
TCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAG
ATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGG
TGCCTCACTGATTAAGCATTGGTAA

WEDNESDAY, 7/6

[Inducible Promoters]

Repeat Colony PCR

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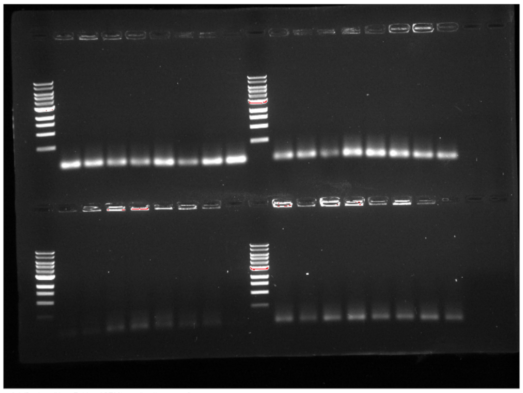
Colony PCR for Screening Recombinant Rhizo Cells with Inducible Promoter_Citrine Constructs /uL				
	S			
	pYU 3143	pYU 3144	pYU 3145	pYU 3147
A	1	9	17	25
B	2	10	18	26
C	3	11	19	27
D	4	12	20	28
E	5	13	21	29
F	6	14	22	30
G	7	15	23	31
neg con	8	16	24	32

Colony PCR for Screening Recombinant Rhizo Cells with Inducible Promoter_Citrine Constructs /uL							
	GC	DMSO	p3143	p3145	Template	dH2O	Total
1x	12.5	0.0	1.3	1.3	negligible	9.9	25.0
40x (32 react	500	0	52	52	#VALUE!	396	1000

Conditions: (1x) 98C for 5min (35x) 98C for 30s, 65C for 15s, 72C for 30s (1x) 72C for 2min, 4C forever

citrine.pdf
p. 1

citrine



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Page 1 of 1

Rif stock solution
Concentration: 100mg/mL

THURSDAY, 7/7

[Constitutive Promoters]
Dark Reader on WC
1/3 of the colonies with Anderson Strong promoters gave off clear citrine fluorescence
no clear fluorescence was observed for either the Anderson medium or the Anderson weak promoter with the naked eye

96 Well Screening for Flourscence

Screen Shot 2016-07-07 at 4.26.58 PM.png

<table>
<tr><th colspan="13">E.coli with RBS_citrine Screening 150uL LB+spec in each well</th></tr>
<tr><td></td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td><td>7</td><td>8</td><td>9</td><td>10</td><td>11</td><td>12</td></tr>
<tr><td>A</td><td colspan="12" rowspan="2">pYU 3155 (identified colonies with flourscence)</td></tr>
<tr><td>B</td></tr>
<tr><td>C</td><td colspan="11" rowspan="3">pYU 3156</td><td></td></tr>
<tr><td>D</td><td></td></tr>
<tr><td>E</td><td>neg con</td></tr>
<tr><td>F</td><td colspan="11" rowspan="3">pYU 3157</td><td></td></tr>
<tr><td>G</td><td></td></tr>
<tr><td>H</td><td>neg con</td></tr>
</table>

left at 34C shaker for incubation overnight
Plates where the colonies were takeing from stored at the Isaacs Lab 4C cold room top shelf
Spec 50 stored at the Isaacs Lab 4C cold room top shelf

Screen Shot 2016-07-10 at 1.08.31 PM.png

	1	2	3	4	5	6	7	8	9	10	11	12	
A	140	67777	64806	59754	63544	67584	45895	58282	67770	68629	63961	72964	Citrine:506, 539
B	58256	59953	66244	76392	68463	69966	45799	66642	72178	40983	46062	72478	Citrine:506, 539
C	159	99	57	102	73	1197	70	369	74	1056	90	91	Citrine:506, 539
D	95	70	105	69	83	979	43	88	83	1228	56	454	Citrine:506, 539
E	93	273	860	1027	81	78	51	1045	76	87	85	102	Citrine:506, 539
F	92	423	99	56	107	1471	89	73	76	2157	72	102	Citrine:506, 539
G	95	88	90	72	92	59	226	73	144	102	79	101	Citrine:506, 539
H	107	94	90	98	83	90	1462	83	76	100	96	99	Citrine:506, 539

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.984	0.998	0.926	0.922	0.934	0.983	0.921	0.84	0.966	0.962	0.972	0.992
B	0.992	0.955	0.956	0.967	0.951	0.929	1.16	0.943	0.973	1.182	1.002	0.974
C	0.958	0.969	1.152	0.953	0.973	0.995	0.959	0.971	0.985	0.987	1.01	0.988
D	0.975	1.028	0.95	0.94	0.897	0.919	1.187	0.928	1.028	0.994	1.205	1.076
E	0.987	1.05	1.047	0.938	1.137	1.053	1.141	0.944	0.99	0.97	0.966	0.964
F	0.986	1.063	1.101	1.265	1.109	1.263	0.999	1.07	1.06	0.999	0.941	0.964
G	0.983	0.959	0.939	0.966	0.95	0.87	0.95	0.866	0.95	0.917	0.911	0.935
H	0.834	0.773	0.885	0.932	1.059	1.13	1.014	0.971	0.987	0.9	0.845	0.09

	1	2	3	4	5	6	7	8	9	10	11	12		
A	142.28	67912.83	69984.88	64809.11	68034.26	68752.80	49831.70	69383.33	70155.28	71339.92	65803.50	73552.42	OD:600	
B	58725.81	62778.01	69292.89	78998.97	71990.54	75313.24	39481.90	70670.20	74180.88	34672.59	45970.06	74412.73	OD:600	red=1
C	165.97	102.17	49.48	107.03	75.03	1203.02	72.99	380.02	75.13	1069.91	89.11	92.11	OD:600	green=2
D	97.44	68.09	110.53	73.40	92.53	1065.29	36.23	94.83	80.74	1235.41	46.47	421.93	OD:600	blue=3
E	94.22	260.00	821.39	1094.88	71.24	74.07	44.70	1106.99	76.77	89.69	87.99	105.81	OD:600	
F	93.31	397.93	89.92	44.27	96.48	1164.69	89.09	68.22	71.70	2159.16	76.51	105.81	OD:600	
G	96.64	91.76	95.85	74.53	96.84	67.82	237.89	84.30	151.58	111.23	86.72	108.02	OD:600	
H	128.30	121.60	101.69	105.15	78.38	79.65	1441.81	85.48	77.00	111.11	113.61	1100.00	OD:600	

20160708 e.coli with new RBS citrine new protocol.xlsx

Cell culture

Picked one colony with Anderson strong promoter (pYU 3155) to make 2 tubes of cell culture, left at 37C shaing for ~5h

Jinny to take out

Next step: miniprep for sequencing; glycerol stock

Meeting with Dr. Moreno tmr

- making glycerol stocks of identified colonies
- minipreping the plasmid for sequencing>>primers: the same set of primers that we used in MCDB 201L for sequencing the promoters???
- designing the primers for modifying plasmid backbones
- two unsuccessful attempt with the colony PCR trying to identify the presence of citrine within the colonies that give off fluorescence

[Inducible Promoters]

96 Well Screening for Flourscence

Screen Shot 2016-07-07 at 4.27.04 PM.png

Rhizo with RBS_citrine Screening 140uL LB+spec in each well 0.5% inducer		
	1 2 3 4 5 6 7 8 9 10 11	12
A	R pYU 3163 7.5 uL Mel	neg con
B	R pYU 3164 7.5 uL Tau	neg con
C	R pYU 3165 7.5 uL Rha	neg con
D	R pYU 3164 7.5 uL Ara	neg con
E	S pYU 3163 7.5 uL Mel	neg con
F	S pYU 3164 7.5 uL Tau	neg con
G	S pYU 3165 7.5 uL Rha	neg con
H	S pYU 3164 7.5 uL Ara	neg con

left at 35C shaker for incubation overnight

the inducers stored at the Isaacs Lab 4C cold room top shelf

Plates where the colonies were takeing from stored at the Isaacs Lab 4C cold room top shelf

Neg con tubes added with 500 uL LB, stored at the Isaacs Lab 4C cold room top shelf

Results:

The 28C shaking incubator tend to dry out the edges of the plate. Use palte film in Natalie's drawer.

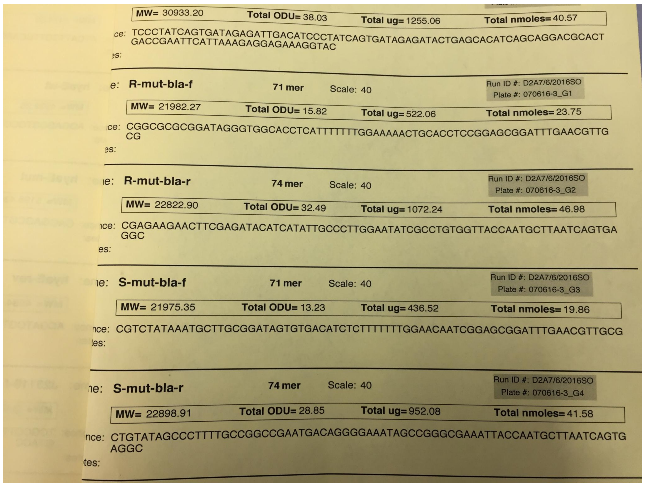
20160708 rhizo wIth citrine.xlsx

[KO MutS]

Primer and Template

The primer order

IMG_2730.JPG



MW= 30933.20 Total ODU= 38.03 Total ug= 1255.06 Total nmoles= 40.57

ce: TCCCTATCAGTGATAGAGATTGACATCCCTATCAGTGATAGAGATACTGAGCACATCAGCAGGACGCACT
GACCGAATTCATTAAAGAGGAGAAAGGTAC

es:

e: **R-mut-bla-f** 71 mer Scale: 40 Run ID #: D2A7/6/2016SO Plate #: 070616-3_G1

MW= 21982.27 Total ODU= 15.82 Total ug= 522.06 Total nmoles= 23.75

ice: CGGCGCGCGGATAGGGTGGCACCTCATTTTTTTGGAAAAACTGCACCTCCGGAGCGGATTTGAACGTTG
CG

es:

ie: **R-mut-bla-r** 74 mer Scale: 40 Run ID #: D2A7/6/2016SO Plate #: 070616-3_G2

MW= 22822.90 Total ODU= 32.49 Total ug= 1072.24 Total nmoles= 46.98

ice: CGAGAAGAACTTCGAGATACATCATATTGCCCTTGGAATATCGCCTGTGGTTACCAATGCTTAATCAGTGA
GGC

es:

ne: **S-mut-bla-f** 71 mer Scale: 40 Run ID #: D2A7/6/2016SO Plate #: 070616-3_G3

MW= 21975.35 Total ODU= 13.23 Total ug= 436.52 Total nmoles= 19.86

nce: CGTCTATAAATGCTTGCGGATAGTGTGACATCTCTTTTTTTGGAACAATCGGAGCGGATTTGAACGTTGCG

tes:

ne: **S-mut-bla-r** 74 mer Scale: 40 Run ID #: D2A7/6/2016SO Plate #: 070616-3_G4

MW= 22898.91 Total ODU= 28.85 Total ug= 952.08 Total nmoles= 41.58

nce: CTGTATAGCCCTTTTGCCGGCCGAATGACAGGGGAAATAGCCGGGCGAAATTACCAATGCTTAATCAGTG
AGGC

tes:

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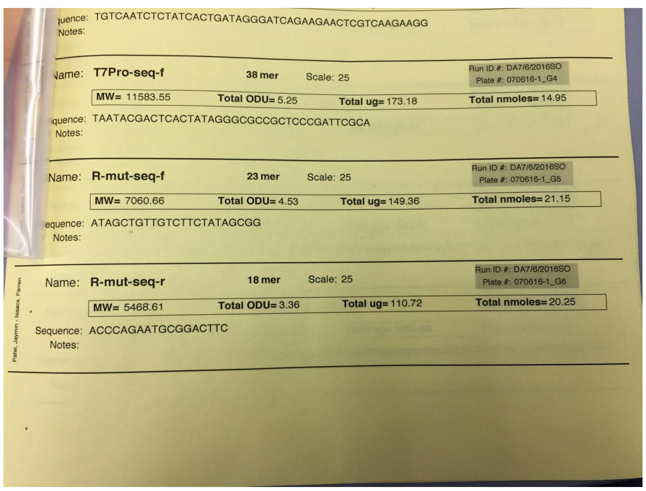


quence: TGTCAATCTCTATCACTGATAGGGATCAGAAGAACTCGTCAAGAAGG

Notes:

Name: **T7Pro-seq-f** 38 mer Scale: 25 Run ID #: DA7/6/2016SO Plate #: 070616-1_G4

MW= 11583.55 Total ODU= 5.25 Total ug= 173.18 Total nmoles= 14.95

quence: TAATACGACTCACTATAGGGCGCCGCTCCCGATTCGCA

Notes:

Name: **R-mut-seq-f** 23 mer Scale: 25 Run ID #: DA7/6/2016SO Plate #: 070616-1_G5

MW= 7060.66 Total ODU= 4.53 Total ug= 149.36 Total nmoles= 21.15

equence: ATAGCTGTTGTCTTCTATAGCGG

Notes:

Name: **R-mut-seq-r** 18 mer Scale: 25 Run ID #: DA7/6/2016SO Plate #: 070616-1_G6

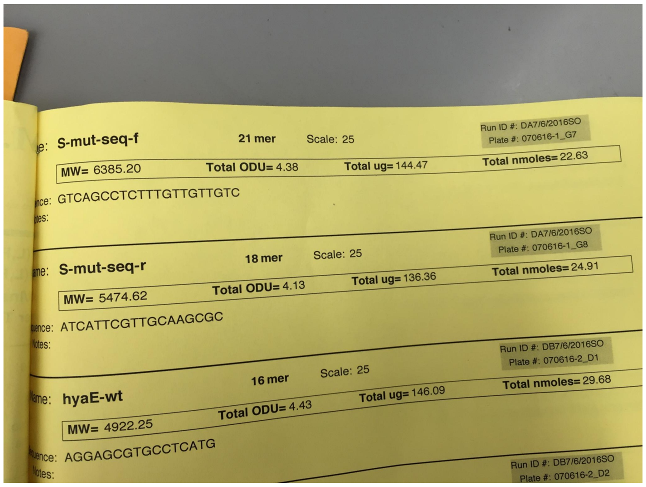
MW= 5468.61 Total ODU= 3.36 Total ug= 110.72 Total nmoles= 20.25

Sequence: ACCCAGAATGCGGACTTC

Notes:

Patel, Jaymin - Isaacs, Farren

IMG_2732.JPG



Stock solution:
Add total # of nmole*5 uL Tris buffer to each primer tube. e.g. 22.63 nmoles>> add 113.15 uL Tris buffer.
Working solution:
Dilute stock solution by 10.

Obtained 100x template from Jaymin, diluted it to 1x. All solutions for PCR stored in -20 in iGEM space.

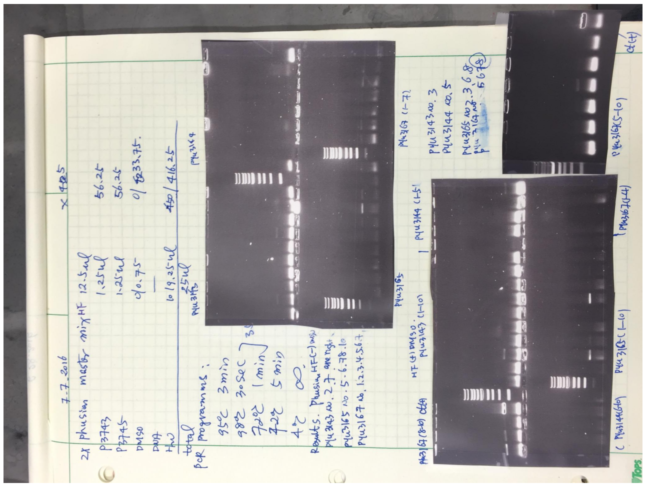
Meeting with Colin on BioBricks

- A plasmid backbone and an insert (whatever you comes up to submit as a part)
- prefix and suffix: iGEM restriction sites
- What kinds of biobricks for the gold medal
- How useful the biobricks are to other teams
- Quality vs. quantity
- Characterization data
- A work flow of BioBricks
- amplify; ligate into vector
- A list of stuff we need on west campus
- [ ] Find out what parts we submitted last year

FRIDAY, 7/8

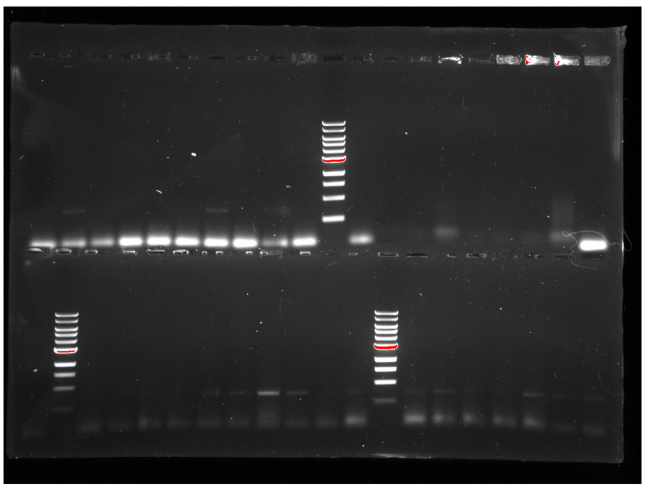
[Inducible Promoters]
Yingchun Repeated the colony PCR with S
Conditions: (1x) 95C for 3min (35x) 98C for 30s, 72C for 1min (1x) 72C for 5min, 4C forever
P3743 and P 3745 MM HF+DMSO

IMG_2734.JPG

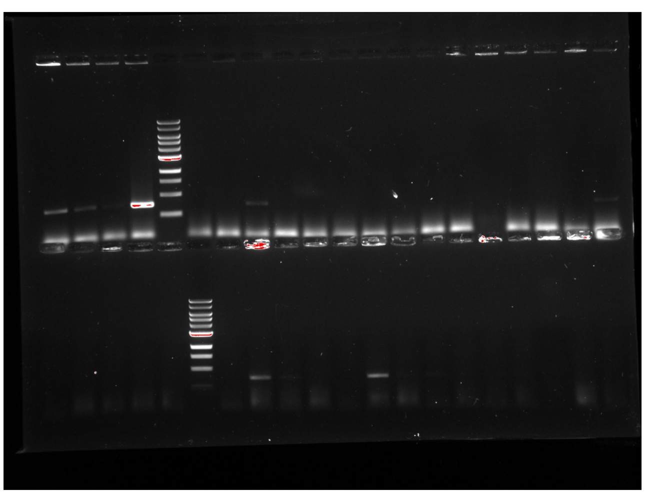


Lane 1-10 pYU 3164; Lane 11-20 pYU 3163; Lane 21-30 pYU 3165; Lane 31-40 pYU 3167

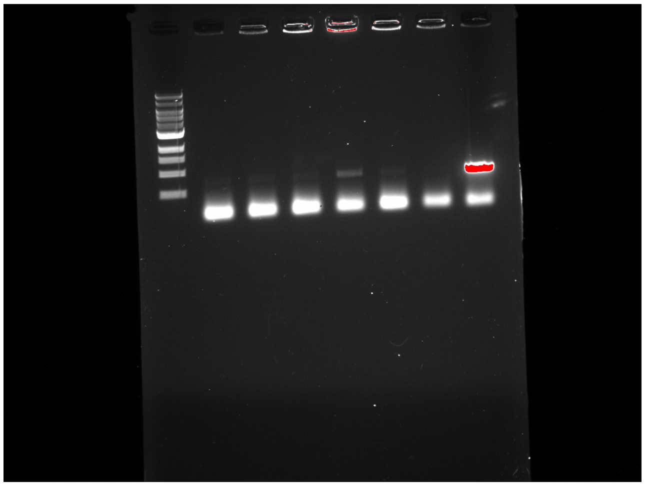
Screen Shot 2016-07-11 at 11.38.18 AM.png



Screen Shot 2016-07-11 at 11.38.52 AM.png



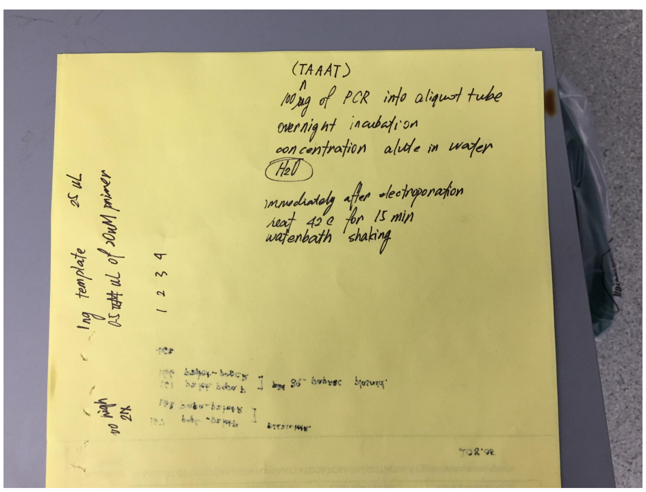
Screen Shot 2016-07-11 at 11.39.03 AM.png



Picked two colonies for each contructs for the 2 strains, striked on plates, grew up in liquid culture
The plates are in the 29.9C incubator in the gel room, the liquid culture are in the 28C shaking incubator in the gel room.

[Constitutive Promoters]
Placed primer order for modifying plasmid backbones.
Jaymin's idea on primer design

IMG_2736.JPG



Here're the primers for modifying plasmid backbones with Anderson promoters. The lower case sequences are promoters, purple RBS, and blue LIC upstream sites.
Anderson Strong:
ttgacggctagctcagtcctaggtacagtgctagcGCAACAAAACAAAAAGTAGAGGAGGTACTTCCAATCCATGGAGACC
Anderson Medium:
ttgacggctagctcagtcctaggtatagtgctagcGCAACAAAACAAAAAGTAGAGGAGGTACTTCCAATCCATGGAGACC
Anderson Weak:
tttatggctagctcagtcctaggtacaatgctagcGCAACAAAACAAAAAGTAGAGGAGGTACTTCCAATCCATGGAGACC

Promoter_LIC_RBS_citrine constructs
Picked 3 colonies for each promoter PiG 346, PiG 347, PiG 348

Table11

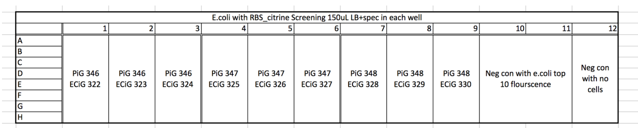
	A	B
1	PiG	
2	346	AStr_LIC_new RBS_citrine
3	347	AMe_LIC_new RBS_citrine
4	348	AWk_LIC_new RBS_citrine

Table12

	A	B	C	D
1	ECiG	PiG		
2	322	346	E. coli Top 10	Spectinomycin (100 mg/L)
3	323	346	E. coli Top 10	Spectinomycin (100 mg/L)
4	324	346	E. coli Top 10	Spectinomycin (100 mg/L)
5	325	347	E. coli Top 10	Spectinomycin (100 mg/L)
6	326	347	E. coli Top 10	Spectinomycin (100 mg/L)
7	327	347	E. coli Top 10	Spectinomycin (100 mg/L)
8	328	348	E. coli Top 10	Spectinomycin (100 mg/L)
9	329	348	E. coli Top 10	Spectinomycin (100 mg/L)
10	330	348	E. coli Top 10	Spectinomycin (100 mg/L)

striked on plates, grew up in liquid culture, set up a 96 well

Screen Shot 2016-07-10 at 1.23.25 PM.png



E.coli with RBS_citrine Screening 150uL LB+spec in each well

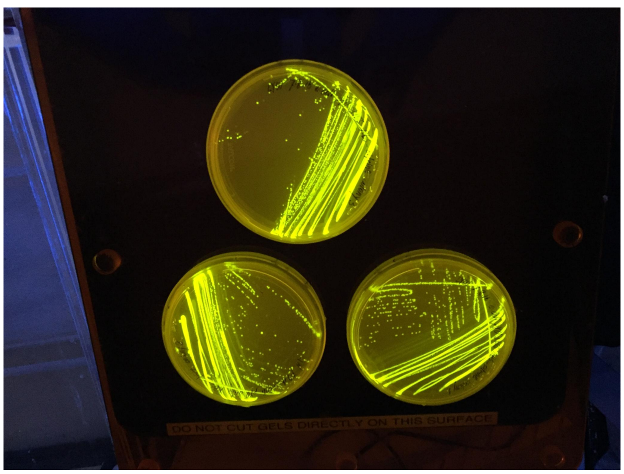
	1	2	3	4	5	6	7	8	9	10	11	12
A B C D E F G H	PiG 346 ECiG 322	PiG 346 ECiG 323	PiG 346 ECiG 324	PiG 347 ECiG 325	PiG 347 ECiG 326	PiG 347 ECiG 327	PiG 348 ECiG 328	PiG 348 ECiG 329	PiG 348 ECiG 330	Neg con with e.coli top 10 flourscence		Neg con with no cells

The plates are in the 37C incubator, the liquid culture and 96 well assay are in the 37C shaking incubator.
Mid log turbidness:

IMG_2735.JPG

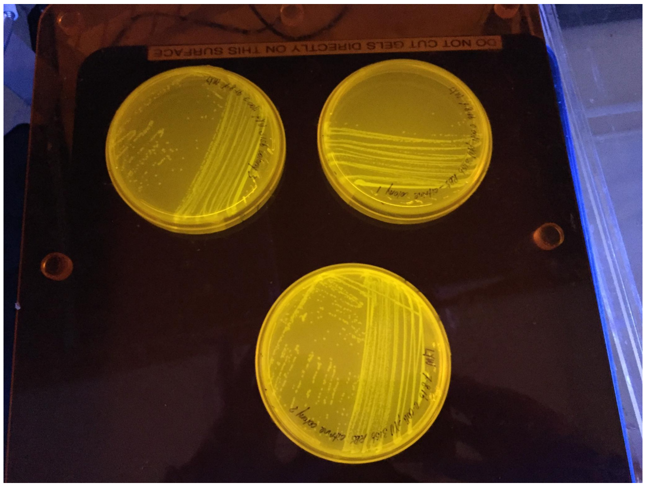
After overnight incubation at 37C:
Anderson Strong:

IMG_2740.JPG



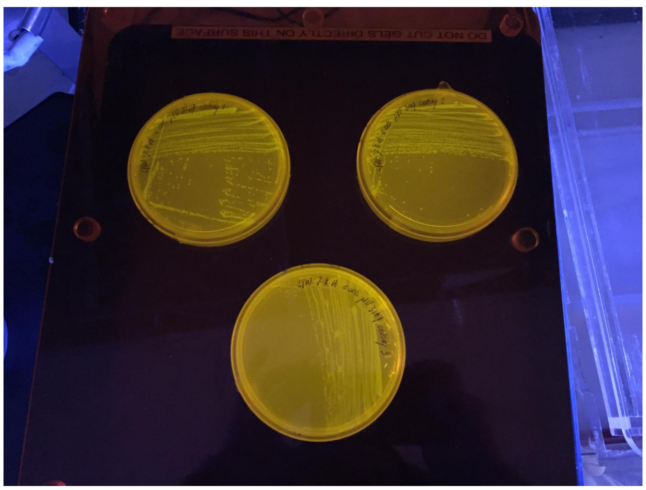
Anderson Medium:

IMG_2739.JPG



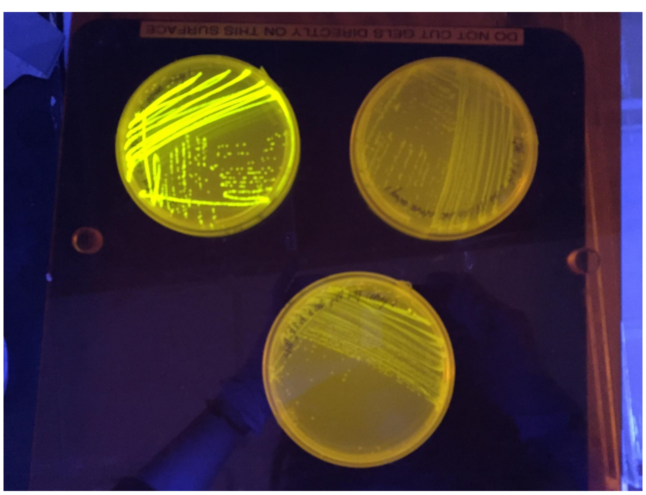
Anderson Weak:

IMG_2738.JPG



Comparison:

IMG_2741.JPG



[KO MutS]

PCR Amplification

Screen Shot 2016-07-10 at 1.28.05 PM.png

PCR Amplification of Constructs to KO MutS in R&S /uL

	R	S	No Primer	No Template	
	1-10	11-20	21-22	23	24
HiFi	12.5	12.5	12.5	12.5	12.5
1x Template	1.0	1.0	1.0	1.0	1.0
R-mut-bla-f	0.5	0.0	0.0	0.5	0.0
R-mut-bla-r	0.5	0.0	0.0	0.5	0.0
S-mut-bla-f	0.0	0.5	0.0	0.0	0.5
S-mut-bla-r	0.0	0.5	0.0	0.0	0.5
dH2O	10.5	10.5	11.5	10.5	10.5

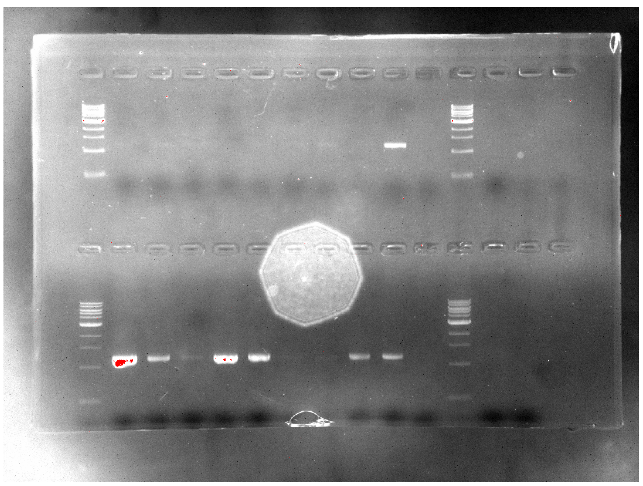
Master Mix /uL

		1x	5x (neg con)	10x
R	HiFi	12.5	62.5	125.0
	1x Template	1.0	5	10.0
	R-mut-bla-f	0.5	2.5	5.0
	R-mut-bla-r	0.5	2.5	5.0
	dH2O	10.5	52.5	105.0
S	HiFi	12.5	62.5	125.0
	1x Template	1.0	5	10.0
	S-mut-bla-f	0.5	2.5	5.0
	S-mut-bla-r	0.5	2.5	5.0
	dH2O	10.5	52.5	105.0

HiFi Master Mix is in Colin's 4C fridge near the bench; both stock and working solutions of primers
Conditions: Default conditions on the PCR machine, changing the annealing time to 5s-10s if the product is smaller than 1kb (HiFi extend within 1s for 1kb)

AGE

20160708 MutS contructs amplification-2.tif



Make Gel in the Isaacs Lab

150 mL for the regular sized gel

- 150 mL 0.5x TBE solution (the big tank next to the sink)
- 1.5g (1%) agarose GPG (on the top of the fridge)
- 15 uL SYBR (10, 000x) or 1.5uL EthBr (100, 000x);

Loading Dye and Ladder both on the rack;

SATURDAY, 7/9

[Constitutive Promoters]

For each tube of 3mL cell culture:

- 500 uL each to make 2 copies of glycerol stocks stored in -80C in iGEM space
- 1500 uL to imniprep for sequencing

Mini Prep (QIAGEN miniprep protocol)

PROM-1206-1095533-HB-QIAprep-Miniprep-0615-WW.pdf

QIAprep-Miniprep-1.jpg

QIAprep Spin

Protocol: Plasmid DNA Purification using the QIAprep Spin Miniprep Kit and a Microcentrifuge

This protocol is designed for purification of up to 20 µg of high-copy plasmid DNA from 1–5 ml overnight cultures of *E. coli* in LB medium. For purification of low-copy plasmids and cosmids, large plasmids (>10 kb), and DNA prepared using other methods, refer to the recommendations on pages 35–36.

Please read "Important Notes" on pages 13–18 before starting.

Note: All protocol steps should be carried out at room temperature (15–25°C).

Procedure

1. **Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.**

   Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet.

   If LyseBlue reagent has been added to Buffer P1, vigorously shake the buffer bottle to ensure LyseBlue particles are completely dissolved. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

2. **Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 4–6 times.**

   Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.

   If LyseBlue has been added to Buffer P1 the cell suspension will turn blue after addition of Buffer P2. Mixing should result in a homogeneously colored suspension. If the suspension contains localized colorless regions or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

3. **Add 350 µl Buffer N3 and mix immediately and thoroughly by inverting the tube 4–6 times.**

   To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer N3. Large culture volumes (e.g. ≥5 ml) may require inverting up to 10 times. The solution should become cloudy.

   If LyseBlue reagent has been used, the suspension should be mixed until all trace of blue has gone and the suspension is colorless. A homogeneous colorless suspension indicates that the SDS has been effectively precipitated.

4. **Centrifuge for 10 min at 13,000 rpm (~17,900 x *g*) in a table-top microcentrifuge.**

   A compact white pellet will form.

20 QIAprep Miniprep Handbook 06/2015

QIAprep-Miniprep-2.jpg

5. **Apply 800 µl of the supernatant from step 4 to the QIAprep 2.0 spin column by pipetting.**
6. **Centrifuge for 30–60 s. Discard the flow-through.**
7. **Recommended: Wash the QIAprep 2.0 spin column by adding 0.5 ml Buffer PB and centrifuging for 30–60 s. Discard the flow-through.**
   This step is necessary to remove trace nuclease activity when using *endA*+ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5®α do not require this additional wash step.
8. **Wash QIAprep 2.0 spin column by adding 0.75 ml Buffer PE and centrifuging for 30–60 s.**
9. **Discard the flow-through, and centrifuge at full speed for an additional 1 min to remove residual wash buffer.**
   Important: Residual wash buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.
10. **Place the QIAprep 2.0 column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of each QIAprep 2.0 spin column, let stand for 1 min, and centrifuge for 1 min.**

QIAprep Spin

QIAprep Miniprep Handbook 06/2015 21

P1 50mL falcon tubes in the first shelf of the 4C fridge next to the sink with MQ water
Others solutions might be on 2 different shelves
Miniprep columns on the shelf above the PCR machine
*For PE and PB buffers, add 400 mL of ethanol whenever staring a new bottle (1 buffer + 4 ethanol)
Use 1-5mL of overnight cell culture; allute the DNA in dH2O

DNA Concentration
After miniprep all 9 tubes are labelled and measured concentration
The machine in between the electroporator and the PCR machines
Add 5uL of sample to the black circle of a magnetic thins
The tubes labelled and stored at the 4C cold room top shelf

MONDAY, 7/11

Monday Meeting

20160711 Week 7 Monday Meeting Slides - Leslie.pptx

PCR techinque: use 3 C below the annealing temperature for the first 10x for colony PCR; regular annealing temperature for the rest

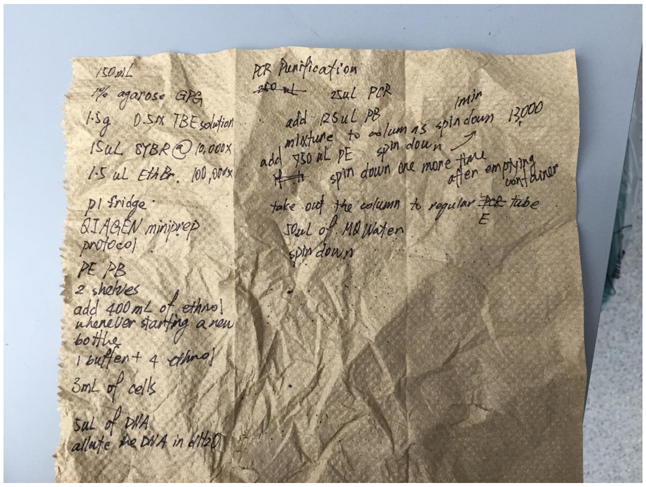
- [ ] Read up on MutS mechanism
- [ ] Meet with Jayin to design the new primers for MutS KO
- [ ] Constitutive promoter plasmid backbone modifications: get rid of the sequence behind LIC upstream

[KO MutS]

PCR Products Purification

Protocol

IMG_2742.JPG



1-10 R 11-20 S 21-24 neg con

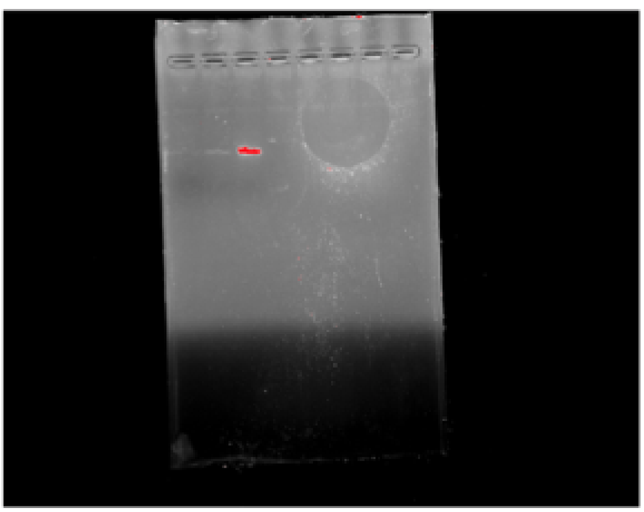
Nanodrop resuts:

–R constructs: 4.0 ug/mL 260/230: 4.00 260/280: N/A

S constrcuts: 36.6 ug/mL 260/230: 2.14 260/280: 1.68

AGE (missing ladder):

clipboard_2016-07-11_14:25:59.png



[Constitutive Promoters]

96 Well Plate Reader

Set-up

Screen Shot 2016-07-10 at 1.23.25 PM.png

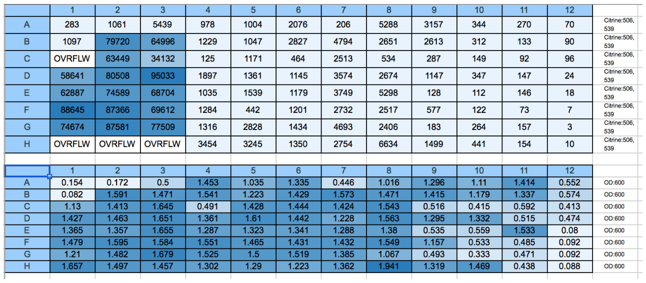
E.coli with RBS_citrine Screening 150uL LB+spec in each well

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D	PiG 346 ECiG 322	PiG 346 ECiG 323	PiG 346 ECiG 324	PiG 347 ECiG 325	PiG 347 ECiG 326	PiG 347 ECiG 327	PiG 348 ECiG 328	PiG 348 ECiG 329	PiG 348 ECiG 330	Neg con with e.coli top 10 flourscence		Neg con with no cells
E												
F												
G												
H												

Results

Data in LB

Screen Shot 2016-07-12 at 5.45.07 AM.png



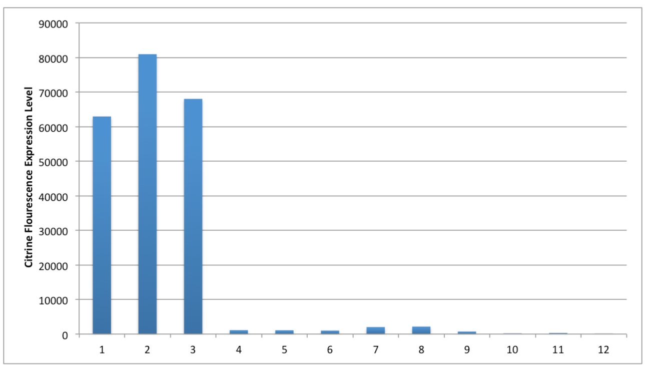
	1	2	3	4	5	6	7	8	9	10	11	12	
A	283	1061	5439	978	1004	2076	206	5288	3157	344	270	70	Citrine:506, 539
B	1097	79720	64996	1229	1047	2827	4794	2651	2613	312	133	90	Citrine:506, 539
C	OVRFLW	63449	34132	125	1171	464	2513	534	287	149	92	96	Citrine:506, 539
D	58641	80508	95033	1897	1361	1145	3574	2674	1147	347	147	24	Citrine:506, 539
E	62887	74589	68704	1035	1539	1179	3749	5298	128	112	146	18	Citrine:506, 539
F	88645	87366	69612	1284	442	1201	2732	2517	577	122	73	7	Citrine:506, 539
G	74674	87581	77509	1316	2828	1434	4693	2406	183	264	157	3	Citrine:506, 539
H	OVRFLW	OVRFLW	OVRFLW	3454	3245	1350	2754	6634	1499	441	154	10	Citrine:506, 539

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.154	0.172	0.5	1.453	1.035	1.335	0.446	1.016	1.296	1.11	1.414	0.552	OD:600
B	0.082	1.591	1.471	1.541	1.223	1.429	1.573	1.471	1.415	1.179	1.337	0.574	OD:600
C	1.13	1.413	1.645	0.491	1.428	1.444	1.424	1.543	0.516	0.415	0.592	0.413	OD:600
D	1.427	1.463	1.651	1.361	1.61	1.442	1.228	1.563	1.295	1.332	0.515	0.474	OD:600
E	1.365	1.357	1.655	1.287	1.323	1.341	1.288	1.38	0.535	0.559	1.533	0.08	OD:600
F	1.479	1.595	1.584	1.551	1.465	1.431	1.432	1.549	1.157	0.533	0.485	0.092	OD:600
G	1.21	1.482	1.679	1.525	1.5	1.519	1.385	1.067	0.493	0.333	0.471	0.092	OD:600
H	1.657	1.497	1.457	1.302	1.29	1.223	1.362	1.941	1.319	1.469	0.438	0.088	OD:600

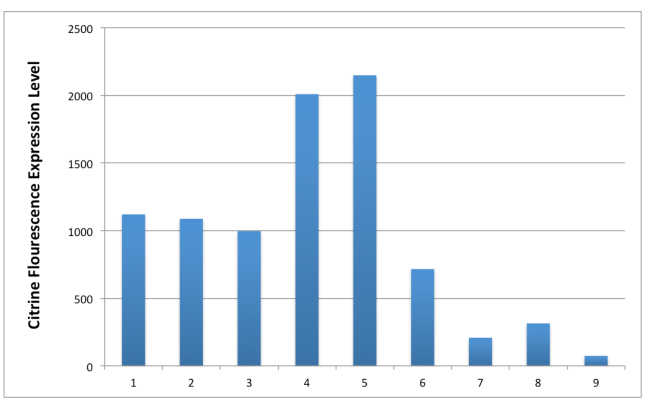
Data in PBS

20160711 E.coli Anderson promoters with LIC_RBS_citrine identified colonies.xlsx

Untitled.png



2.png



Put the miniprepped plasmid in -20C!

[Inducible Promoters]

Assigned ECiG #

Screen Shot 2016-07-12 at 5.37.51 AM.png

331		R. tropicii	Spectinomycin (100 mg/L)	WC	11-Jul-16	Leslie Wang	pYU 3163 colony D
332		R. tropicii	Spectinomycin (100 mg/L)	WC	11-Jul-16	Leslie Wang	pYU 3163 colony F
333		R. tropicii	Spectinomycin (100 mg/L)	WC	11-Jul-16	Leslie Wang	pYU 3164 colony B
334		R. tropicii	Spectinomycin (100 mg/L)	WC	11-Jul-16	Leslie Wang	pYU 3164 colony E
335		R. tropicii	Spectinomycin (100 mg/L)	WC	11-Jul-16	Leslie Wang	pYU 3165 colony D
336		R. tropicii	Spectinomycin (100 mg/L)	WC	11-Jul-16	Leslie Wang	pYU 3165 colony G
337		R. tropicii	Spectinomycin (100 mg/L)	WC	11-Jul-16	Leslie Wang	pYU 3167 colony E
338		R. tropicii	Spectinomycin (100 mg/L)	WC	11-Jul-16	Leslie Wang	pYU 3167 colony F
339		S. meliloti	Spectinomycin (100 mg/L)	WC	11-Jul-16	Leslie Wang	pYU 3163 colony 2
340		S. meliloti	Spectinomycin (100 mg/L)	WC	11-Jul-16	Leslie Wang	pYU 3163 colony 7
341							
342		S. meliloti	Spectinomycin (100 mg/L)	WC	11-Jul-16	Leslie Wang	pYU 3164 colony 5
343		S. meliloti	Spectinomycin (100 mg/L)	WC	11-Jul-16	Leslie Wang	pYU 3165 colony 5
344		S. meliloti	Spectinomycin (100 mg/L)	WC	11-Jul-16	Leslie Wang	pYU 3165 colony 6
345		S. meliloti	Spectinomycin (100 mg/L)	WC	11-Jul-16	Leslie Wang	pYU 3167 colony 6

Unable to find 2 colony culture for S. meliloti pYU3164 and pYU 3167. 2 lanes left blank for potential addition to the library

96 Well plates

Screen Shot 2016-07-12 at 5.35.58 AM.png

<table>
<tr><td colspan="13">R Rhizo with RBS_citrine Screening 125uL LB+spec in each well 10uL (0.75%) inducer</td></tr>
<tr><td></td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td><td>7</td><td>8</td><td>9</td><td>10</td><td>11</td><td>12</td></tr>
<tr><td>A</td><td colspan="11">R ECiG 331 pYU 3163-D 10 uL Mel</td><td>neg con</td></tr>
<tr><td>B</td><td colspan="11">R ECiG 332 pYU 3163-F 10 uL Mel</td><td>neg con</td></tr>
<tr><td>C</td><td colspan="11">R ECiG 333 pYU 3164-B 10 uL Tau</td><td>neg con</td></tr>
<tr><td>D</td><td colspan="11">R ECiG 334 pYU 3164-E 10 uL Tau</td><td>neg con</td></tr>
<tr><td>E</td><td colspan="11">R ECiG 335 pYU 3165-D 10 uL Rha</td><td>neg con</td></tr>
<tr><td>F</td><td colspan="11">R ECiG 336 pYU 3165-G 10 uL Rha</td><td>neg con</td></tr>
<tr><td>G</td><td colspan="11">R ECiG 337 pYU 3167-E 10 uL Ara</td><td>neg con</td></tr>
<tr><td>H</td><td colspan="11">R ECiG 338 pYU 3167-F 10 uL Ara</td><td>neg con</td></tr>
</table>

<table>
<tr><td colspan="13">S Rhizo with RBS_citrine Screening 125uL LB+spec in each well 10uL (0.75%) inducer</td></tr>
<tr><td></td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td><td>7</td><td>8</td><td>9</td><td>10</td><td>11</td><td>12</td></tr>
<tr><td>A</td><td colspan="11">S ECiG 339 pYU 3163-2 10 uL Mel</td><td>neg con</td></tr>
<tr><td>B</td><td colspan="11">S ECiG 340 pYU 3163-7 10 uL Mel</td><td>neg con</td></tr>
<tr><td>C</td><td colspan="11">S ECiG 341 pYU 3164-5 10 uL Tau</td><td>neg con</td></tr>
<tr><td>D</td><td colspan="11">S ECiG 341 pYU 3164-5 10 uL Tau</td><td>neg con</td></tr>
<tr><td>E</td><td colspan="11">S ECiG 342 pYU 3165-5 10 uL Rha</td><td>neg con</td></tr>
<tr><td>F</td><td colspan="11">S ECiG 343 pYU 3165-6 10 uL Rha</td><td>neg con</td></tr>
<tr><td>G</td><td colspan="11">S ECiG 344 pYU 3167-6 10 uL Ara</td><td>neg con</td></tr>
<tr><td>H</td><td colspan="11">S ECiG 344 pYU 3167-6 10 uL Ara</td><td>neg con</td></tr>
</table>

TUESDAY, 7/12

[Constitutive Promoters]

Transformed PiG 346-1 and PiG 346-2 in both R and S (2 reactions each, labelled A and B)
1uL of 25 ng/mL plasmid + 20 uL competent cells
Plated on spec+suc, 28C incubator in OML

Updated Primer Orders
Anderson Strong:
ttgacggctagctcagtcctaggtacagtgctagcGCAACAAAACAAAAAGTAGAGGAGGTACTTCCAATCCATG
Anderson Medium:
ttgacggctagctcagtcctaggtatagtgctagcGCAACAAAACAAAAAGTAGAGGAGGTACTTCCAATCCATG
Anderson Weak:
tttatggctagctcagtcctaggtacaatgctagcGCAACAAAACAAAAAGTAGAGGAGGTACTTCCAATCCATG

WEDNESDAY, 7/13

[Constitutive Promoters]

Dr. Moreno says the original primers order was correct bc of the extra bps are necessary for a annealing temperature close to 60C
Jaymin will reorder them

[Inducible Promoters]

Flat-line results for citrine expression assay. Maybe a dead-end

Screen Shot 2016-07-15 at 11.36.57 AM.png

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.741	0.958	0.919	1.418	1.022	1.043	1.009	0.991	1.114	1.022	1.131	0.088	OD:600
B	0.999	0.985	1.015	1.086	1.085	1.061	1.159	1.029	1.193	1.344	1.063	0.09	OD:600
C	0.765	0.718	0.688	0.669	0.729	0.764	0.765	1.46	0.658	0.65	0.745	0.088	OD:600
D	0.809	0.677	0.67	0.681	0.6	0.659	0.604	0.759	0.576	0.652	0.629	0.088	OD:600
E	1.397	1.376	1.397	1.369	1.41	1.412	1.394	1.421	1.422	1.433	1.404	0.087	OD:600
F	1.498	1.5	1.518	1.497	1.486	1.51	1.489	1.478	1.519	1.505	1.485	0.089	OD:600
G	0.605	0.7	0.619	0.501	0.75	0.564	0.198	0.597	0.598	0.615	0.629	0.086	OD:600
H	1.018	1.451	0.725	0.678	1.026	0.772	0.647	0.64	0.148	0.682	0.596	0.087	OD:600

	1	2	3	4	5	6	7	8	9	10	11	12	
A	172	206	206	115	191	139	161	116	186	195	151	109	Citrine:506, 539
B	174	178	188	134	159	136	185	144	173	81	152	92	Citrine:506, 539
C	159	169	154	163	137	115	118	61	112	150	118	92	Citrine:506, 539
D	129	146	156	131	125	136	121	120	125	121	114	84	Citrine:506, 539
E	165	172	183	158	144	137	135	139	151	136	179	109	Citrine:506, 539
F	136	116	143	129	145	131	117	130	157	122	127	83	Citrine:506, 539
G	103	144	128	108	110	105	28	103	122	112	106	89	Citrine:506, 539
H	124	71	123	116	102	130	126	107	110	111	117	88	Citrine:506, 539

	1	2	3	4	5	6	7	8	9	10	11	12
A	232	215	224	81	187	133	160	117	167	191	134	1239
B	174	181	185	123	147	128	160	140	145	60	143	1022
C	208	235	224	244	188	151	154	42	170	231	158	1045
D	159	216	233	192	208	206	200	158	217	186	181	955
E	118	125	131	115	102	97	97	98	106	95	127	1253
F	91	77	94	86	98	87	79	88	103	81	86	933
G	170	206	207	216	147	186	141	173	204	182	169	1035
H	122	49	170	171	99	168	195	167	743	163	196	1011

20160713 S. meliloti with citrine in PBS.xlsx

[KO MutS]

Primer design for KO MutS & MutL in rhizo by natural recombination

Primer List for KO MutS:L in rhizo by natural recombineering.xlsx

Screen Shot 2016-07-14 at 10.47.13 AM.png

priG	primer name	sequence	Note
414	Kan-Amp-F	TCGGCAAGGTGTTCTGG	Red= Kanamycin homology
415	Kan-Amp-R	GATCTGAATTGCTATGTTTAGTGAGTT	
416	R-MutS-US-F	TGATTTCCGGATCGGGATTG	
417	R-MutS-US-R	CTATGCGCCGACCAGAACACCTTGCCGCCGCTATAGAAGACAACAG	
418	R-MutS-DS-F	CTCACTAAACATAGCAATTCAGATCCGATATTCCAAGGGCAATATGATG	
419	R-MutS-DS-R	CCGATATGACAGTGCGCA	
420	R-MutL-US-F	GCCGTCACGCGCAAT	
421	R-MutL-US-R	CTATGCGCCGACCAGAACACCTTGCCACCCGTGCTTTGTTGAGA	
422	R-MutL-DS-F	CTCACTAAACATAGCAATTCAGATCTCCGGAAAATAGGCTGGAAG	
423	R-MutL-DS-R	AGATTGCGATCGACTATGCC	
424	S-MutS-US-F	TCTTCGGGCGTGATTTCC	
425	S-MutS-US-R	CTATGCGCCGACCAGAACACCTTGCGAGATGTCACACTATCCGCA	
426	S-MutS-DS-F	CTCACTAAACATAGCAATTCAGATCTTCGCCCGGCTATTTCC	
427	S-MutS-DS-R	TGCCGGTCAGGAAATGC	
428	S-MutL-US-F	ATCCGACCGTTCGAAGTG	
429	S-MutL-US-R	CTATGCGCCGACCAGAACACCTTGCTCCTATTTTCCAGGATTCGGAG	
430	S-MutL-DS-F	CTCACTAAACATAGCAATTCAGATCGAACCTGCCGGAACCATATT	
431	S-MutL-DS-R	ATGTTCGTCTTCGTCCAGG	
432	R-MutS-seq-F	GCCATTGTTCCTCCAAACC	
433	R-MutS-seq-r	GAAGCGCTCACCCAGAAT	
434	R-MutL-seq-f	GCAACCTGTCATCATTCCCT	
435	R-MutL-seq-r	ATGACGCGTAAAGATCGCT	
436	S-MutS-seq-f	GATACCCGGCATGTTTCCT	
437	S-MutS-seq-r	GACTTAGATCATTCGTTGCAAGC	
438	S-MutL-seq-f	TACCGGCCGACAGCTAT	
439	S-MutL-seq-r	TGAACGTCCTGATCTATCGTG	

R-MutL.ape

R-MutS.ape

resistance cassette from portMAGE2.ape.ape

S-MutL.ape

S-MutS.ape

THURSDAY, 7/14

[KO MutS]

Primer design for KO MutS & MutL in rhizo by natural recombination

IMG_2761.JPG

W.M. Keck Oligonucleotide Synthesis Facility

Yale University School of Medicine

What's new? ⇒⇒⇒ *NEW LOW-COST SYNTHESIS SCALE: 25 nmole desalted for normal DNA oligos <65 bases*

Name (L,F): Patel, Jaymin
PI's name (L,F): Isaacs, Farren
Dept/Inst: FASMCB Administration
Deliver To: West Campus, ISTC Building, Room 319

Order date: 7/12/2016
Oligos in Order: 10
Phone #: 215-260-2031
Billing: 1070521.1.Q00042.833620.615002

Patel, Jaymin - Isaacs, Farren

Name: **Kan-Amp-F** **18 mer** Scale: 25 Run ID #: DB7/12/2016JO Plate #: 071216-2_F5

MW= 5561.67 **Total ODU=** 3.03 **Total ug=** 99.99 **Total nmoles=** 17.98

Sequence: TCGGCAAGGTGTTCTGGT 89.9uL

Notes:

Patel, Jaymin - Isaacs, Farren

Name: **Kan-Amp-R** **24 mer** Scale: 25 Run ID #: DB7/12/2016JO Plate #: 071216-2_F6

MW= 7406.91 **Total ODU=** 4.51 **Total ug=** 148.67 **Total nmoles=** 20.07

Sequence: TGAGTAAACTTGGTCTGACAGTTA 100.35 uL

Notes:

Patel, Jaymin - Isaacs, Farren

Name: **R-MtS-US-F** **19 mer** Scale: 25 Run ID #: DB7/12/2016JO Plate #: 071216-2_F7

MW= 5877.88 **Total ODU=** 7.62 **Total ug=** 251.33 **Total nmoles=** 42.76

Sequence: GCCATGGCGAAGATGATCG 213.8 uL

Notes:

Patel, Jaymin - Isaacs, Farren

Name: **R-MtS-US-R** **47 mer** Scale: 25 Run ID #: DB7/12/2016JO Plate #: 071216-2_F8

MW= 14457.43 **Total ODU=** 9.43 **Total ug=** 311.32 **Total nmoles=** 21.53

Sequence: TGCGCCGACCAGAACACCTTGCCGAGGAGGTGCAGTTTTTCCAAAAA 107.65uL

Notes:

Patel, Jaymin - Isaacs, Farren

Name: **R-MtS-DS-F** **45 mer** Scale: 25 Run ID #: DB7/12/2016JO Plate #: 071216-2_F9

MW= 13798.03 **Total ODU=** 13.19 **Total ug=** 435.34 **Total nmoles=** 31.55

Sequence: GTAACTGTCAGACCAAGTTTACTCACCACAGGCGATATTCCAAGG 157.75uL

Notes:

IMG_2762.JPG

Name: R-MtS-DS-R 18 mer Scale: 25 Run ID #: DB7/12/2016JO Plate #: 071216-2_F10

MW= 5531.63 Total ODU= 3.76 Total ug= 124.21 Total nmoles= 22.45

Sequence: GACTTGGCCTCGCGGATG 112.23 uL

Notes:

Name: S-MtS-US-F 18 mer Scale: 25 Run ID #: DB7/12/2016JO Plate #: 071216-2_F11

MW= 5595.69 Total ODU= 4.14 Total ug= 136.59 Total nmoles= 24.41

Sequence: GATCGGGATTGGCCATGG 122.05 uL

Notes:

Name: S-MtS-US-R 51 mer Scale: 25 Run ID #: DB7/12/2016JO Plate #: 071216-2_F12

MW= 15670.24 Total ODU= 20.12 Total ug= 663.96 Total nmoles= 42.37

Sequence: TGCGCCGACCAGAACACCTTGCCGAGATTGTTCCAAAAAAAGAGATGTCAC 211.85 uL

Notes:

Name: S-MtS-DS-F 43 mer Scale: 25 Run ID #: DB7/12/2016JO Plate #: 071216-2_G1

MW= 13079.54 Total ODU= 11.25 Total ug= 371.15 Total nmoles= 28.38

Sequence: GTAACTGTCAGACCAAGTTTACTCATTTCGCCCGGCTATTTCC 141.9 uL

Notes:

Name: S-MtS-DS-R 18 mer Scale: 25 Run ID #: DB7/12/2016JO Plate #: 071216-2_G2

MW= 5467.57 Total ODU= 3.70 Total ug= 121.94 Total nmoles= 22.30

Sequence: TCCGCCTTGCCGGTCAGG 111.5 uL

Notes:

FRIDAY, 7/15

[Constitutive Promoters]

Brought over the [LYW 7.12.16 rhizo with piG 346-1/2-A/B] plates from OML

No observable fluorescence under the drak reader

Picked one single colonies from each plate to set up liquid culture [LYW 7.15.16 rhizo with piG 346-1/2-A/B] in 28C shaking in cubator

[KO MutS]

PCR Amplification of Parts of Constructs

*HiFi is in the -20C fridge in the room next to the gel room

Screen Shot 2016-07-15 at 11.41.07 AM.png

PCR ampification for parts of KO MutS in rhizo by natural recombination /uL

	Kan resistance cassette			R-MtS-US			R-MtS-DS			S-MtS-DS			S-MtS-DS		
	prG 414, prG 415, PiG301			prG 416, prG 417, wt R			prG 418, prG 419, wt R			prG 424, prG425, wt S			prG 426, prG 427, wt S		
	pos	no primer	no temp	pos	no primer	no temp	pos	no primer	no temp	pos	no primer	no temp	pos	no primer	no temp
	1-6	7	8	9-14	15	16	17-22	23	24	25-30	31	32	33-38	39	40
HiFi	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
1x Template	1.0	1.0	0.0	1.0	1.0	0.0	1.0	1.0	0.0	1.0	1.0	0.0	1.0	1.0	0.0
F-Primer	0.5	0.0	0.5	0.5	0.0	0.5	0.5	0.0	0.5	0.5	0.0	0.5	0.5	0.0	0.5
R-Primer	0.5	0.0	0.5	0.5	0.0	0.5	0.5	0.0	0.5	0.5	0.0	0.5	0.5	0.0	0.5
dH2O	10.5	11.5	11.5	10.5	11.5	11.5	10.5	11.5	11.5	10.5	11.5	11.5	10.5	11.5	11.5

Master Mix /uL

		1x	7x			1x	7x
Kan res	HiFi	12.5	87.5	S-MtS-US	HiFi	12.5	87.5
	dH2O	10.5	73.5		dH2O	10.5	73.5
	PiG 301	1.0	7.0		PiG 301	1.0	7.0
	prG 414	0.5	3.5		prG 414	0.5	3.5
	prG 415	0.5	3.5		prG 415	0.5	3.5
R-MtS-US	HiFi	12.5	87.5	S-MtS-DS	HiFi	12.5	87.5
	dH2O	10.5	73.5		dH2O	10.5	73.5
	PiG 301	1.0	7.0		PiG 301	1.0	7.0
	prG 414	0.5	3.5		prG 414	0.5	3.5
	prG 415	0.5	3.5		prG 415	0.5	3.5
R-MtS-DS	HiFi	12.5	87.5			1x	12x
	dH2O	10.5	73.5	neg con	HiFi	12.5	150.0
	PiG 301	1.0	7.0		dH2O	11.5	138.0
	prG 414	0.5	3.5				
	prG 415	0.5	3.5				

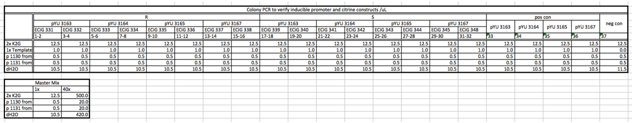
PCR conditions

(1x) 95C for 5min (32x) 98C for 20s, 60C for 15s, 72C for 1min (1x) 72C for 3min, 4C forever

[Inducible Promoters]

Colony PCR for rhizo transformed with inducible promoters and citrine constructs

Screen Shot 2016-07-18 at 10.35.57 AM.png



Colony PCR to verify inducible promoter and citrine constructs /uL

	R							
	pYU 3163		pYU 3164		pYU 3165		pYU 3167	
	ECiG 331	ECiG 332	ECiG 333	ECiG 334	ECiG 335	ECiG 336	ECiG 337	ECiG 338
	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16
2x K2G	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
1x Template	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
p 1130 from	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
p 1131 from	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
dH2O	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5

	S								pos con				neg con
	pYU 3163		pYU 3164		pYU 3165		pYU 3167		pYU 3163	pYU 3164	pYU 3165	pYU 3167	
	ECiG 339	ECiG 340	ECiG 341	ECiG 342	ECiG 343	ECiG 344	ECiG 345	ECiG 348					
	17-18	19-20	21-22	23-24	25-26	27-28	29-30	31-32	33	34	35	36	37
2x K2G	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
1x Template	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0
p 1130 from	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
p 1131 from	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
dH2O	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	11.5

Master Mix		
	1x	40x
2x K2G	12.5	500.0
p 1130 from	0.5	20.0
p 1131 from	0.5	20.0
dH2O	10.5	420.0

PCR Conditions:

[Rhzio Spec Resistance]

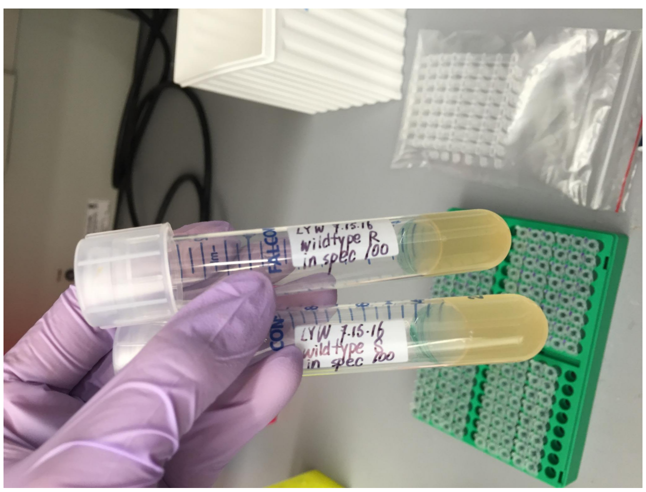
Started 1 liquid culture of both wildtype R and wildtype S, left in the shaking incubator over the weekend

MONDAY, 7/18

[Rhzio Spec Resistance]

Growth observed in both culture tubes

IMG_2774.JPG



Verify spec resistance with control

Got e.coli strain from Natalie that's not spec resistant as control

[LYW 7.18.16 wildtype R in spec 100] [LYW 7.18.16 wildtype S in spec 100] in 28C shaking incubator

neg con: [LYW 7.18.16 e.coli from NJM in spec 100] in 28C shaking incubator

pos con: [LYW 7.18.16 wildtype R] [LYW 7.18.16 wildtype S] [LYW 7.18.16 e.coli from NJM] in 28C shaking incubator

Colony PCR to verify strains

Conditions:

ID PCRs on gel contaminants - template boil

- Goal: to figure out if the stuff in the gelboxes is prokaryotic or eukaryotic. There may be more than one thing in there, but hopefully there's one dominant thing that I can sequence.
- Template: (insert your templates here, including if they're solid or liquid culture and what dilution factor was -- 1:20 is standard)
- Primers:
    - 16s: 940 and 941

- ITS: 942 and 943

* Program: METHOD CALC || HOTLID 105, 30 || VOLUME 25 || TEMP 95.0, 180 || TEMP 98.0, 20 || TEMP**57, 15** || TEMP **72.0, 75** || GOTO: 2, 26 || TEMP 72.0, 300 || TEMP 12.0, 0 || END
* 25uL rxns
* Used K2G Hifi

Set-up:

Screen Shot 2016-07-18 at 3.56.59 PM.png

Colony PCR to verify strains

	wildtype R from 7.15.16 liquid culture		wildtype S from 7.15.16 liquid culture		wildtype R from comptent cell tube		wildtype S from comptent cell tube		R with PZP spec from 7.8.16 liquid culture	
	pr941 pr946	pr942 pr943	pr941 pr946	pr942 pr943	pr941 pr946	pr942 pr943	pr941 pr946	pr942 pr943	pr941 pr946	pr942 pr943
	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20
HiFi	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
1x Template	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
F-Primer	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
R-Primer	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
dH2O	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5

	S with PZP spec from 7.8.16 liquid culture		R with PZP spec from 7.12.16 plates		S with PZP spec from plates		neg con	
	pr941 pr946	pr942 pr943	pr941 pr946	pr942 pr943	pr941 pr946	pr942 pr943	pr941 pr946	pr942 pr943
	21-22	23-24	25-26	27-28	29-30	31-32	33	34
HiFi	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
1x Template	1.0	1.0	1.0	1.0	1.0	1.0	0.0	0.0
F-Primer	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
R-Primer	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
dH2O	10.5	10.5	10.5	10.5	10.5	10.5	11.5	11.5

Master Mix /uL

		1x	20x
pr941 pr946	HiFi	12.5	250.0
	dH2O	10.5	210.0
	PiG 301	1.0	20.0
	pr941	0.5	10.0
	pr946	0.5	10.0
pr942 pr943	HiFi	12.5	250.0
	dH2O	10.5	210.0
	PiG 301	1.0	20.0
	pr942	0.5	10.0
	pr943	0.5	10.0

[KO MutS]

Screen Shot 2016-07-18 at 10.35.57 AM.png

Colony PCR to verify inducible promoter and citrine constructs /uL

	R								S		
	pYU 3163		pYU 3164		pYU 3165		pYU 3167		pYU 3163		pYU 3164
	ECiG 331	ECiG 332	ECiG 333	ECiG 334	ECiG 335	ECiG 336	ECiG 337	ECiG 338	ECiG 339	ECiG 340	ECiG 341
	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20	21-22
2x K2G	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
1x Template	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
p 1130 from	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
p 1131 from	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
dH2O	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5

	S						pos con				neg con
	pYU 3164	pYU 3165		pYU 3167			pYU 3163	pYU 3164	pYU 3165	pYU 3167	
	ECiG 342	ECiG 343	ECiG 344	ECiG 345	ECiG 348						
	23-24	25-26	27-28	29-30	31-32		33	34	35	36	37
2x K2G	12.5	12.5	12.5	12.5	12.5		12.5	12.5	12.5	12.5	12.5
1x Template	1.0	1.0	1.0	1.0	1.0		1.0	1.0	1.0	1.0	0.0
p 1130 from	0.5	0.5	0.5	0.5	0.5		0.5	0.5	0.5	0.5	0.5
p 1131 from	0.5	0.5	0.5	0.5	0.5		0.5	0.5	0.5	0.5	0.5
dH2O	10.5	10.5	10.5	10.5	10.5		10.5	10.5	10.5	10.5	11.5

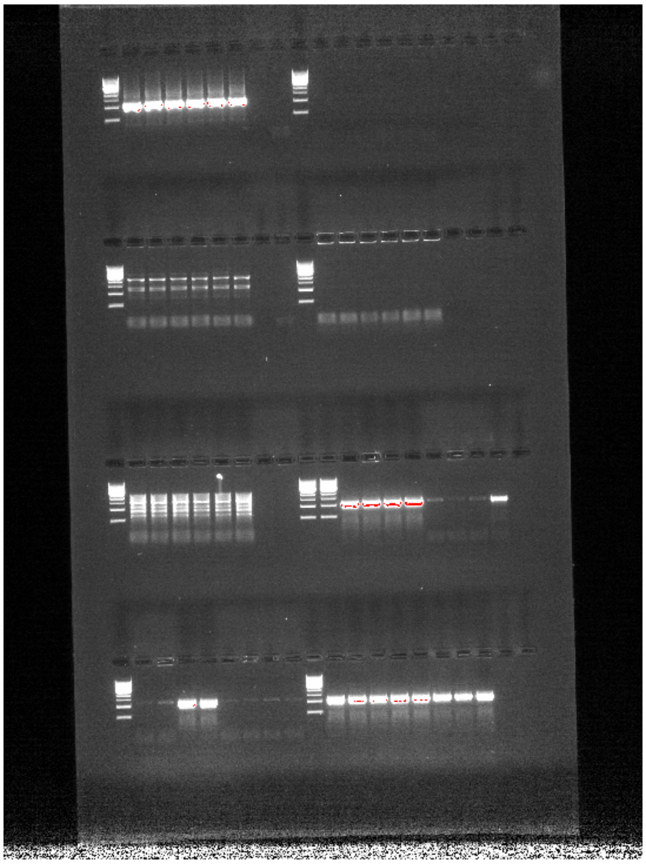
Master Mix

	1x	40x
2x K2G	12.5	500.0
p 1130 from	0.5	20.0
p 1131 from	0.5	20.0
dH2O	10.5	420.0

Gel Results for Colony PCR (1uL orange loading dye+5uL of PCR reaction)

Gel loaded as 1 column of 1kb ladder followed by 8 reactions

20160718 KO MtS + Colony of Rhizo.tif



Kan cassette is fine. Double check the primers. Miniprep?

WEDNESDAY, 7/20

Outreach Meeting:
3-5 professor
a theme?
Dani Keller
stipend $1200
experiment.com: video?

Colony PCR to verify Anderson strong promoter and citrine constructs /uL

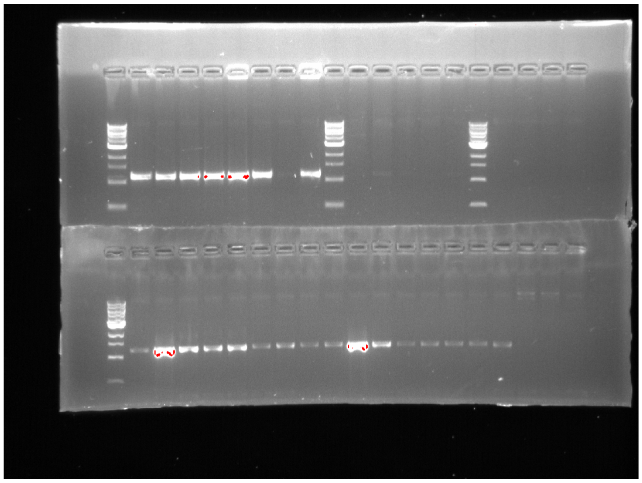
Screen Shot 2016-07-21 at 10.41.16 AM.png

Colony PCR to verify Anderson strong promoter and citrine constructs /uL

	R				S				piG 346-1	piG 346-2	neg cob
	piG 346-1-A	piG 346-1-B	piG 346-2-A	piG 346-2-B	piG 346-1-A	piG 346-1-B	piG 346-2-A	piG 346-2-B			
	1, 9	2, 10	3, 11	4, 12	5, 13	6, 14	7, 15	8, 16	17	18	19
2x K2G	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
1x Template	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0
p 1130 from	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
p 1131 from	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
dH2O	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	11.5

Master Mix		
	1x	20x
2x K2G	12.5	250.0
p 1130 from	0.5	10.0
p 1131 from	0.5	10.0
dH2O	10.5	210.0

20160720 colony of rhizo.tif



Run-2 PCR ampification for parts of KO MutS in rhizo by natural recombination

Screen Shot 2016-07-21 at 10.41.31 AM.png

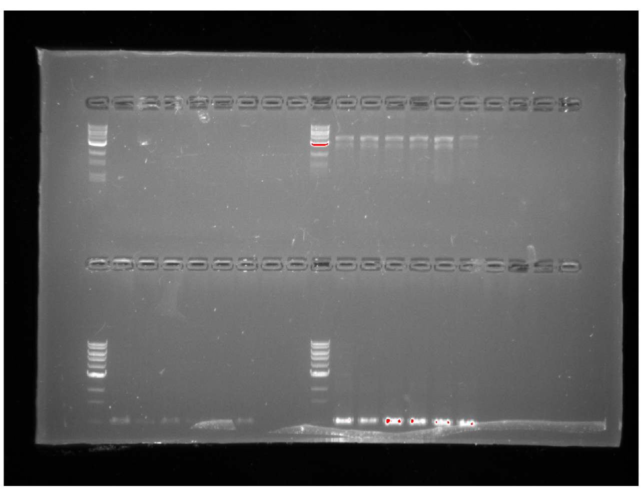
Run-2 PCR ampification for parts of KO MutS in rhizo by natural recombination /uL

	R-MtS-US			R-MtS-DS			S-MtS-DS			S-MtS-DS		
	prG 416, prG 417, wt R			prG 418, prG 419, wt R			prG 424, prG425, wt S			prG 426, prG 427, wt S		
	pos	no primer	no temp	pos	no primer	no temp	pos	no primer	no temp	pos	no primer	no temp
	1-6	7	8	9-14	15	16	17-22	23	24	25-30	31	32
HiFi	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
1x Template	1.0	1.0	0.0	1.0	1.0	0.0	1.0	1.0	0.0	1.0	1.0	0.0
F-Primer	0.5	0.0	0.5	0.5	0.0	0.5	0.5	0.0	0.5	0.5	0.0	0.5
R-Primer	0.5	0.0	0.5	0.5	0.0	0.5	0.5	0.0	0.5	0.5	0.0	0.5
butane	10.5	11.5	11.5	10.5	11.5	11.5	10.5	11.5	11.5	10.5	11.5	11.5

Master Mix /uL

		1x	7x			1x	7x			1x	9x
R-MtS-US	HiFi	12.5	87.5	S-MtS-US	HiFi	12.5	87.5	neg con	HiFi	12.5	112.5
	butane	10.5	73.5		butane	10.5	73.5		butane	11.5	103.5
	PiG 301	1.0	7.0		PiG 301	1.0	7.0	overall	HiFi	12.5	462.5
	prG 416	0.5	3.5		prG 424	0.5	3.5		butane	11.5	425.5
	prG 417	0.5	3.5		prG 425	0.5	3.5				
R-MtS-DS	HiFi	12.5	87.5	S-MtS-DS	HiFi	12.5	87.5				
	butane	10.5	73.5		butane	10.5	73.5				
	PiG 301	1.0	7.0		PiG 301	1.0	7.0				
	prG 418	0.5	3.5		prG 426	0.5	3.5				
	prG 419	0.5	3.5		prG 427	0.5	3.5				

20160720 KO MutS buting.tif



THURSDAY, 7/21

96 Well Assay - One last time

Screen Shot 2016-07-21 at 10.41.39 AM.png

<table>
<tr><th colspan="13">R Rhizo with RBS_citrine Screening 125uL LB+spec in each well 10uL (0.75%) inducer</th></tr>
<tr><th></th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th><th>7</th><th>8</th><th>9</th><th>10</th><th>11</th><th>12</th></tr>
<tr><td>A</td><td rowspan="8">R ECiG 331 pYU 3163-D 7.5 uL 10% Mel</td><td rowspan="8">R ECiG 335 pYU 3165-D 7.5 uL 10% Rha</td><td rowspan="8">R ECiG 337 pYU 3167-E 7.5 uL 10% Ara</td><td rowspan="8">S ECiG 339 pYU 3163-2 7.5 uL 10% Mel</td><td rowspan="8">S ECiG 342 pYU 3165-5 7.5 uL 10% Rha</td><td rowspan="8">S ECiG 344 pYU 3167-6 7.5 uL 10% Ara</td><td rowspan="8">R ECiG 349 piG 346-1-A</td><td rowspan="8">S ECiG 353 piG 346-1-A</td><td rowspan="8">R ECiG 333 pYU 3164-B 25 uL 1.2% Tau</td><td rowspan="8">S ECiG 341 pYU 3164-5 25 uL 1.2 %Tau</td><td rowspan="4">E. coli ECiG 322 piG 346-1</td><td rowspan="2">R wt</td></tr>
<tr><td>B</td></tr>
<tr><td>C</td><td rowspan="2">S wt</td></tr>
<tr><td>D</td></tr>
<tr><td>E</td><td rowspan="4">E. coli ECiG 325 piG 347-1</td><td rowspan="4">LB</td></tr>
<tr><td>F</td></tr>
<tr><td>G</td></tr>
<tr><td>H</td></tr>
</table>

Miniprepping Genomic DNA

1.jpg

Gram-Negative Bacteria

Protocol: Pretreatment for Gram-Negative Bacteria

This protocol is designed for purification of total DNA from Gram-negative bacteria, such as *E. coli*. The protocol describes the preliminary harvesting of bacteria before DNA purification.

Important points before starting

- See "Quantification of starting material", page 17, for details of how to collect and store samples, and how to determine the number of cells in a bacterial culture.
- This pretreatment protocol has not been thoroughly tested and optimized for high-throughput DNA purification using the DNeasy 96 Blood & Tissue Kit. As a general guideline, we recommend to decrease the amount of starting material when using this protocol with the DNeasy 96 Blood & Tissue Kit.

Procedure

1. **Harvest cells (maximum 2×10^9 cells) in a microcentrifuge tube by centrifuging for 10 min at 5000 x *g* (7500 rpm). Discard supernatant.**
2. **Resuspend pellet in 180 µl Buffer ATL.**
3. **Continue with step 2 of the protocol "Purification of Total DNA from Animal Tissues (Spin-Column Protocol)", page 29.**

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2.jpg

We strongly recommend to cut the tissue into small pieces to enable more efficient lysis. If desired, lysis time can be reduced by grinding the sample in liquid nitrogen* before addition of Buffer ATL and proteinase K. Alternatively, tissue samples can be effectively disrupted before proteinase K digestion using a rotor–stator homogenizer, such as the QIAGEN TissueRuptor, or a bead mill, such as the QIAGEN TissueLyser (see page 56 for ordering information). A supplementary protocol for simultaneous disruption of up to 48 tissue samples using the TissueLyser can be obtained by contacting QIAGEN Technical Services (see back cover).

For rodent tails, a maximum of 1.2 cm (mouse) or 0.6 cm (rat) tail should be used. When purifying DNA from the tail of an adult mouse or rat, it is recommended to use only 0.4–0.6 cm.

Animal Tissues (Spin-Column Protocol)

2. **Add 20 µl proteinase K. Mix thoroughly by vortexing, and incubate at 56°C until the tissue is completely lysed. Vortex occasionally during incubation to disperse the sample, or place in a thermomixer, shaking water bath, or on a rocking platform.**

Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1–3 h or, for rodent tails, 6–8 h. If it is more convenient, samples can be lysed overnight; this will not affect them adversely.

After incubation the lysate may appear viscous, but should not be gelatinous as it may clog the DNeasy Mini spin column. If the lysate appears very gelatinous, see the "Troubleshooting Guide", page 47, for recommendations.

Optional: If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml), mix by vortexing, and incubate for 2 min at room temperature before continuing with step 3.

Transcriptionally active tissues such as liver and kidney contain high levels of RNA, which will copurify with genomic DNA. For tissues that contain low levels of RNA, such as rodent tails, or if residual RNA is not a concern, RNase A digestion is not necessary.

3. **Vortex for 15 s. Add 200 µl Buffer AL to the sample, and mix thoroughly by vortexing. Then add 200 µl ethanol (96–100%), and mix again thoroughly by vortexing.**

It is essential that the sample, Buffer AL, and ethanol are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution. Buffer AL and ethanol can be premixed and added together in one step to save time when processing multiple samples.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

DNeasy Blood & Tissue Handbook 07/2006 29

3.jpg

Animal Tissues (Spin-Column Protocol)

A white precipitate may form on addition of Buffer AL and ethanol. This precipitate does not interfere with the DNeasy procedure. Some tissue types (e.g., spleen, lung) may form a gelatinous lysate after addition of Buffer AL and ethanol. In this case, vigorously shaking or vortexing the preparation is recommended.

4. **Pipet the mixture from step 3 (including any precipitate) into the DNeasy Mini spin column placed in a 2 ml collection tube (provided). Centrifuge at ≥6000 x *g* (8000 rpm) for 1 min. Discard flow-through and collection tube.***
5. **Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW1, and centrifuge for 1 min at ≥6000 x *g* (8000 rpm). Discard flow-through and collection tube.***
6. **Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW2, and centrifuge for 3 min at 20,000 x *g* (14,000 rpm) to dry the DNeasy membrane. Discard flow-through and collection tube.**

   It is important to dry the membrane of the DNeasy Mini spin column, since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during the following elution.

   Following the centrifugation step, remove the DNeasy Mini spin column carefully so that the column does not come into contact with the flow-through, since this will result in carryover of ethanol. If carryover of ethanol occurs, empty the collection tube, then reuse it in another centrifugation for 1 min at 20,000 x g (14,000 rpm).
7. **Place the DNeasy Mini spin column in a clean 1.5 ml or 2 ml microcentrifuge tube (not provided), and pipet 200 µl Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at ≥6000 x *g* (8000 rpm) to elute.**

   Elution with 100 µl (instead of 200 µl) increases the final DNA concentration in the eluate, but also decreases the overall DNA yield (see Figure 2, page 21).
8. **Recommended: For maximum DNA yield, repeat elution once as described in step 7.**

   This step leads to increased overall DNA yield.

   A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, to combine the eluates, the microcentrifuge tube from step 7 can be reused for the second elution step.

   Note: Do not elute more than 200 µl into a 1.5 ml microcentrifuge tube because the DNeasy Mini spin column will come into contact with the eluate.

* Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach. See page 8 for safety information.

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Overlapping PCR Assemble S-US, Kan Cassette, S-DS

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	Molecular W	Concentration		Volumn of 10 nmol	
	Da	ug/mL	nmol/mL	uL	
Kan cassette	329522	202	6140	1.62	
R-US	309612				
R-DS	309281				
S-US	310537	36	1151	8.69	
S-DS	305965	304	9924	1.01	

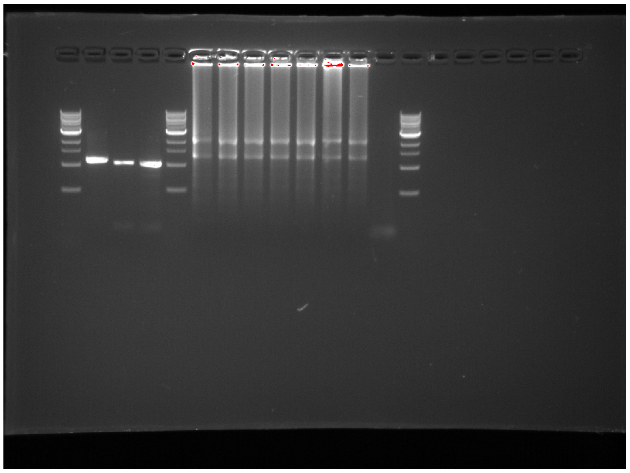
Overlapping PCR Assemble S-US, Kan Cassette, S-DS			
	1x (1-7)	8x	neg con
HiFi	12.5	100.0	12.5
piG 424	0.6	4.8	0.6
piG 427	0.6	4.8	0.6
Kan cassette	1.6	12.8	0.0
S-US	8.7	69.5	0.0
S-DS	1.0	8.0	0.0
dH2O	0.0	0.0	11.3

PCR Conditions: (1x) 95C for 5min (35x) 98C for 20s, 59C-67C gradient for 15s, 72C for 3min (1x) 72C for 5min, 4C forever

AGE:

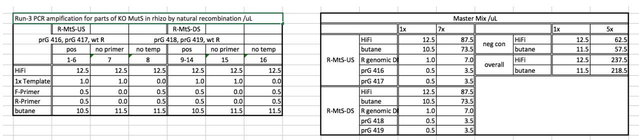
First 3 lanes: purified constructs (1-Kan cassette, 2-S-MtS-US, 3-S-Mts-DS)

20160722 S MtS KO overlapping PCR.tif



Run-3 PCR ampification for parts of KO MutS in rhizo by natural recombination

Screen Shot 2016-07-24 at 3.54.35 PM.png

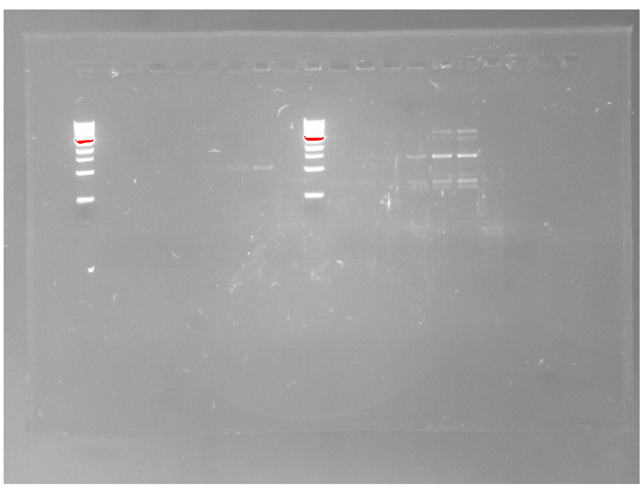


Run-3 PCR ampification for parts of KO MutS in rhizo by natural recombination /uL						
	R-MtS-US			R-MtS-DS		
	prG 416, prG 417, wt R			prG 418, prG 419, wt R		
	pos	no primer	no temp	pos	no primer	no temp
	1-6	7	8	9-14	15	16
HiFi	12.5	12.5	12.5	12.5	12.5	12.5
1x Template	1.0	1.0	0.0	1.0	1.0	0.0
F-Primer	0.5	0.0	0.5	0.5	0.0	0.5
R-Primer	0.5	0.0	0.5	0.5	0.0	0.5
butane	10.5	11.5	11.5	10.5	11.5	11.5

Master Mix /uL							
		1x	7x			1x	5x
R-MtS-US	HiFi	12.5	87.5	neg con	HiFi	12.5	62.5
	butane	10.5	73.5		butane	11.5	57.5
	R genomic D	1.0	7.0	overall	HiFi	12.5	237.5
	prG 416	0.5	3.5		butane	11.5	218.5
	prG 417	0.5	3.5				
R-MtS-DS	HiFi	12.5	87.5				
	butane	10.5	73.5				
	R genomic D	1.0	7.0				
	prG 418	0.5	3.5				
	prG 419	0.5	3.5				

PCR Conditions: (1x) 95C for 3min (35x) 98C for 20s, 58C-70C gradient for 15s, 72C for 30s (1x) 72C for 5min, 4C forever

20160724 genomic DNA KO-MtS for R-US and DS.tif



SUNDAY, 7/24

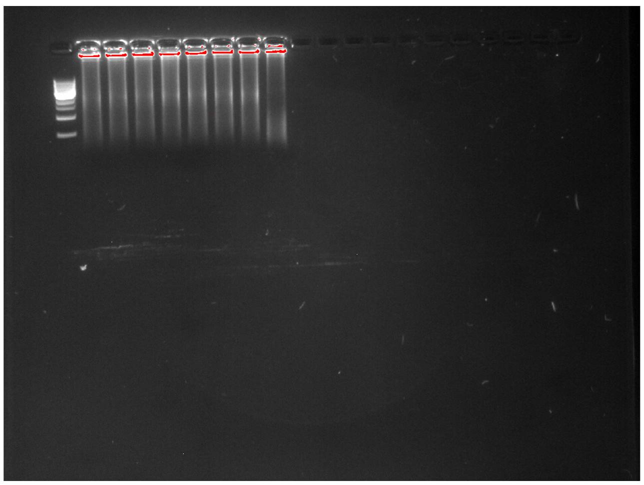
Run-2 Overlapping PCR Assemble S-US, Kan Cassette, S-DS

Screen Shot 2016-07-24 at 3.56.15 PM.png

Run-2 Overlapping PCR Assemble S-US, Kan			
	1x (1-7)	8x	neg con
HiFi	12.5	100.0	12.5
piG 424	0.6	4.8	0.6
piG 427	0.6	4.8	0.6
PCR product	1.0	8.0	0.0
betaing	10.3	82.4	11.3

PCR Conditions: (1x) 98C for 30s (34x) 98C for 10s, 58C-65C gradient for 20s, 72C for 90s (1x) 72C for 5min, 4C forever

2.tif



Run-4 PCR ampification for parts of KO MutS in rhizo by natural recombination

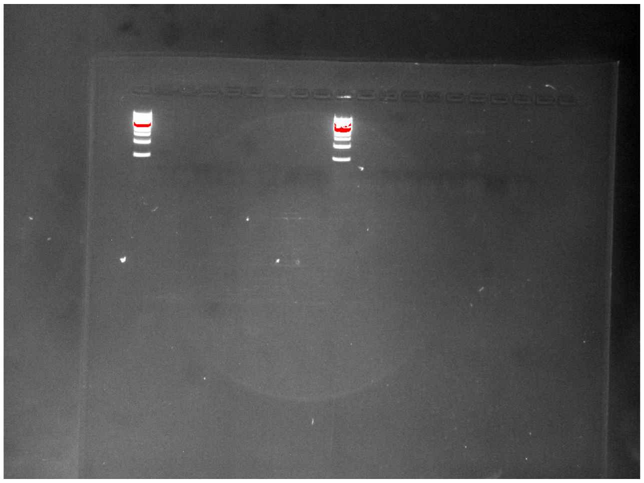
Screen Shot 2016-07-24 at 3.53.28 PM.png

Run-4 PCR ampification for parts of KO MutS in rhizo by natural recombination /uL						
	R-MtS-US			R-MtS-DS		
	prG 416, prG 417, wt R			prG 418, prG 419, wt R		
	pos	no primer	no temp	pos	no primer	no temp
	1-6	7	8	9-14	15	16
HiFi	12.5	12.5	12.5	12.5	12.5	12.5
1x Template	1.0	1.0	0.0	1.0	1.0	0.0
F-Primer	0.5	0.0	0.5	0.5	0.0	0.5
R-Primer	0.5	0.0	0.5	0.5	0.0	0.5
butane	10.5	11.5	11.5	10.5	11.5	11.5

Master Mix /uL							
		1x	7x			1x	5x
R-MtS-US	HiFi	12.5	87.5	neg con	HiFi	12.5	62.5
	butane	10.5	73.5		butane	11.5	57.5
	R genomic DI	1.0	7.0	overall	HiFi	12.5	237.5
	prG 416	0.5	3.5		butane	11.5	218.5
	prG 417	0.5	3.5				
R-MtS-DS	HiFi	12.5	87.5				
	butane	10.5	73.5				
	R genomic DI	1.0	7.0				
	prG 418	0.5	3.5				
	prG 419	0.5	3.5				

PCR Conditions: (1x) 95C for 3min (26x) 98C for 20s, 65C-68C gradient for 15s, 72C for 30s (1x) 72C for 5min, 4C forever

1.tif



Repeat (see next day):

PCR Conditions: (1x) 95C for 3min (26x) 98C for 20s, 59C for 15s, 72C for 30s (1x) 72C for 5min, 4C forever

MONDAY, 7/25

[7/25 PCR 1] Run-5 PCR ampification for parts of KO MutS in rhizo by natural recombination

Screen Shot 2016-07-24 at 3.53.28 PM.png

Run-4 PCR ampification for parts of KO MutS in rhizo by natural recombination /uL						
	R-MtS-US			R-MtS-DS		
	prG 416, prG 417, wt R			prG 418, prG 419, wt R		
	pos	no primer	no temp	pos	no primer	no temp
	1-6	7	8	9-14	15	16
HiFi	12.5	12.5	12.5	12.5	12.5	12.5
1x Template	1.0	1.0	0.0	1.0	1.0	0.0
F-Primer	0.5	0.0	0.5	0.5	0.0	0.5
R-Primer	0.5	0.0	0.5	0.5	0.0	0.5
butane	10.5	11.5	11.5	10.5	11.5	11.5

Master Mix /uL							
		1x	7x			1x	5x
R-MtS-US	HiFi	12.5	87.5	neg con	HiFi	12.5	62.5
	butane	10.5	73.5		butane	11.5	57.5
	R genomic D	1.0	7.0	overall	HiFi	12.5	237.5
	prG 416	0.5	3.5		butane	11.5	218.5
	prG 417	0.5	3.5				
R-MtS-DS	HiFi	12.5	87.5				
	butane	10.5	73.5				
	R genomic D	1.0	7.0				
	prG 418	0.5	3.5				
	prG 419	0.5	3.5				

* same reactions, different PCR conditions than Run-4

PCR Conditions: (1x) 95C for 3min (26x) 98C for 20s, 59C for 15s, 72C for 30s (1x) 72C for 5min, 4C forever

[7/25 PCR 2] Step-by-step Ovrlp PCR Assemble Kan Cassette, S-DS

Screen Shot 2016-07-25 at 10.30.43 AM.png

Step-by-step Ovrlp PCR Assemble Kan Cassette, S-DS			
	DS-kan		neg con
	1x (1-7)	8x	8
HiFi	12.5	100.0	12.5
piG 424	0.0	0.0	0.0
piG 415	0.0	0.0	0.0
piG 414	0.5	4.0	0.5
piG 427	0.5	4.0	0.5
Kan cassette	1.6	12.8	0
S-US	0.0	0.0	0
S-DS	1.0	8.0	0
dH2O	8.9	71.2	11.5

PCR Conditions: (1x) 95C for 3min (26x) 98C for 20s, 59C for 70s, 72C for 30s (1x) 72C for 5min, 4C forever

[7/25 PCR 3]